NUTRITIONAL MODULATION OF IMMUNITY AND PHYSIOLOGICAL RESPONSES IN BEEF CALVES

A Thesis

by

MICHAEL WAYNE KURZ

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

August 2004

Major Subject: Nutrition

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Approved as to style and content by:

Gordon E. Carstens (Chair of Committee)	Thomas H. Welsh (Member)
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ABSTRACT

Nutritional Modulation of Immunity and Physiological Responses

in Beef Calves. (August 2004)

Michael Wayne Kurz, B.S., Texas A&M University

Chair of Advisory Committee: Dr. Gordon E. Carstens

Twenty-four crossbred steer calves (235 $\pm$ 6 kg initial BW) were weaned, adapted to a growing diet, trained to use Calan feeders and assigned to one of four treatments in a 2 x 2 factorial arrangement as follows: (1) no transit stress and saline injection, (2) no transit stress and trace mineral and vitamin E (TM/VitE) injections, (3) transit stress and saline injection and (4) transit stress and TM/VitE injection. TM/VitE injections consisted of Multimin^{TM} to provide 0.3, 0.6, 0.15 and 0.6 mg of Cu, Mn, Se, and Zn/kg BW, respectively, and Vita E^{TM} to provide 9 IU of VitE/kg BW on d 0 and 21 of the study. On d 24, transit-stressed steers were subjected to a three-d, 2000-km, transportation period. During the 56-d experiment, liver and serum samples were assayed to assess trace mineral status, antibody titers to keyhole limpet hemocyanin (KLH) measured to assess humoral immunity and phytohemagglutinin(PHA)- and ovalbumin(OVA)-induced skin swelling and lymphocyte proliferation measured to assess cellular immunity. TM/VitE-injected steers grew slower ($P < 0.05$) over the first 24 d of the experiment and tended ($P = 0.10$) to grow slower after transit. TM/VitE injections increased ($P < 0.05$) liver Cu and Zn concentrations 135 and 21 % by d 30, respectively. TM/VitE-injected steers had increased ($P < 0.05$) serum Cu on d 24 and

26, but decreased ($P < 0.05$) serum Zn and whole blood Se compared to saline-injected steers on d 24 and 56. TM/VitE injections increased serum α-tocopherol concentrations. TM/VitE injections did not affect humoral immunity, but decreased ($P < 0.05$) skin-swelling responses to PHA on d 21, and decreased ($P < 0.05$) lymphocyte proliferation to OVA and KLH. Transit-stressed steers had higher ($P < 0.05$) serum Cu on d 26, and higher ($P < 0.05$) liver Cu concentrations on d 56 than non-transit-stressed steers. Transit stress did not affect serum Zn or whole blood Se concentrations. Injections of TM/VitE increased Cu, Zn and vitamin E, but not Se status. The TM/VitE treatment suppressed growth and cellular immune function suggesting that levels of trace minerals used in this study were too high.

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CHAPTER I

INTRODUCTION

In order to achieve efficient growth and production of beef cattle, it is important to maintain herd health through proper nutrition and handling. Because most of today's beef cattle are finished in a feedlot not in close proximity to the original ranch, transit stress is an important factor, which may affect the production and economic value of cattle. Transit is an intense stressor for most cattle that involves handling, hauling, food and water deprivation, and sometimes changes in climate. Stressful conditions can impair an animal's ability to fight disease challenges. The release of glucocorticcoids, such as cortisol, from the adrenal medulla has been known to reduce immune function by reducing lymphocyte proliferation, and decreasing cytokine production.

Stressful conditions also induce an increase in oxidative processes that lead to production of a highly reactive group of compounds called reactive oxygen metabolites (ROM). Reactive oxygen metabolites are also normal products of metabolism and have important physiologic roles such as in the respiratory burst of phagocytic cells during bacterial degradation. Antioxidant mechanisms are present in the body and are supported by essential nutrients such as copper (Cu), zinc (Zn), selenium (Se), manganese (Mn), vitamin E, and vitamin A. Some of these nutrients are cofactors for enzymes that reduce ROM, such as glutathione peroxidase (GSH-Px), copper-zinc superoxide dismutase (Cu/Zn SOD) and manganese superoxide dismutase (Mn SOD).

This thesis follows the style and format of *Journal of Animal Science*.

Dietary trace mineral levels needed for optimal immune function are often times higher than the levels recommended for maximal growth or prevention of clinical deficiencies. Certain minerals, such as S, Mo and Fe, have the ability to inhibit the effectiveness of dietary Cu, Zn, Se, and Mn by binding them in the rumen thus rendering them unavailable for absorption in the small intestine. The use of an injectable trace mineral supplement eliminates this inhibition by providing Cu, Zn, Se, and Mn directly to the tissue. The goal of this experiment is to determine the effect that antioxidant injection and/or transit stress will have on performance and immune function in beef calves.

CHAPTER II

LITERATURE REVIEW

Copper Absorption and Metabolism

The essentiality of copper (Cu) as a nutrient was established in the 1920s when Hart et al. (1928) demonstrated that supplementation with both iron (Fe) and Cu was required to correct anemia in milk-fed rats. By the 1930s researchers discovered that cattle and sheep raised on Cu-deficient soils in Florida had high incidences of "salt sickness" that was remedied by Cu supplementation (Neal and Scott, 1931). These early discoveries led to the general acceptance of Cu as an essential nutrient for most living organisms.

Copper is poorly absorbed by ruminants (Underwood, 1977), with a mere 5 to 10% of total dietary copper being absorbed by adults (Symonds and Forbes, 1993). Although Cu can be absorbed throughout the entire intestinal tract, the primary site of absorption is the small intestine. Bronner and Yost (1985) proposed that Cu absorption takes place by a two-step process including both active transport and simple diffusion. The protein metallothionine in the mucosal lining of digestive tract epithelium has been shown to bind Cu, and production of metallothionine has been suggested as a mechanism of regulation of Cu absorption (Fisher et al., 1981). Fisher et al. (1981) found that copper absorption was decreased in response to high dietary zinc (Zn). While metallothionine is produced in response to both Cu and Zn, the latter induces synthesis to a greater extent (Oestreicher and Cousins, 1985) and is thought to be responsible for prevention of Cu toxicity in all ruminants except sheep (Cousins, 1985). Fisher et al.

(1981) hypothesized that Zn induced synthesis of metallothionine and that Cu entering the mucosal cell displaced Zn from metallothionine. Since Cu was more tightly bound to metallothionine than Zn, the Cu was rendered unavailable for transfer out of the cell thereby decreasing its ability to diffuse into the portal blood. This Cu, trapped in the mucosal cells, was then sloughed off to become part of the intestinal contents once more.

Once Cu has been actively transported into the mucosal cells of the small intestine, it diffuses into the blood where it binds loosely to the serum protein albumin and some amino acids, such as histidine, for transport throughout the body. The primary storage site for Cu in cattle is the liver with concentrations ranging from 25 to 100 ppm DM in normal adults. From the liver, a metalloprotein called cerruloplasmin (Cp) acts as a carrier to deliver Cu to target cells. Wirth and Linder (1985) found that Cp is a globular protein, which contains 6 to 8 atoms of Cu and accounts for 80 to 90% of total blood Cu. In most species, bile is the primary excretory route for Cu, although small amounts may be excreted through urine, milk and feces (Underwood, 1977).

Antagonists of Copper Absorption

Understanding the antagonistic effects of inorganic constituents such as sulfur (S), molybdenum (Mo), and Iron (Fe), are key factors to consider in Cu absorption, storage, and transfer. These metals can influence the Cu status of an animal shifting it from deficient levels to toxic levels during consumption of an all-natural diet (Suttle, 1991). Copper deficiencies are classified as being either primary or secondary. Primary deficiencies result from inadequate intake of dietary Cu, while secondary deficiencies result from ingestion of adequate levels of dietary Cu in combination with high levels of

antagonists. Hardt et al. (1993) reported that 95 and 99% of bermudagrass and native pastures, respectively, in Texas contained ≤ 10 ppm Cu DM. Concentrations within a range of 5 to 10 ppm are considered adequate for beef cattle when no antagonists are present in the diet, however this situation rarely exists. Perhaps more interesting was the finding that more than 50 and 35% of bermudagrass forage samples contained levels of S (0.32 to 0.67%) and Fe (> 200 ppm), respectively, which have been implicated in reducing Cu absorption. Numerous studies have been conducted to evaluate the effects of S, Mo, and Fe on Cu status in calves (Genglebach et al., 1997; Wang et al., 1988; Bremner et al., 1987; Boyne and Arthur, 1986; Humphries et al., 1985). Bremner et al. (1987) reported that Friesian steer calves fed high-Fe diets exhibited decreased liver Cu concentrations, which decreased further on supplementation with dietary S. These authors also reported that the cattle fed a supplement containing both Fe and Mo showed the largest decrease in liver Cu concentration.

Sulfur, in the absence of Mo, may induce a secondary Cu deficiency by forming insoluble Cu sulfide in the rumen; however, when high levels of S and Mo are present, sulfate is reduced to sulfide, which reacts with Mo to form thiomolybdate (MoS_4). Thiomolybdates bind Cu rendering it unavailable for absorption into the portal system. The changes that ensue effect equilibrium between the compartments and cause Cu to move into the plasma, thus depleting hepatic stores (Wang et al., 1988). Studies by Suttle (1974) and Suttle and Field (1974) suggest that the antagonistic effects of S and Mo on Cu absorption take place in the rumen. When Mo was supplemented in the diet and Cu administered by subcutaneous (s.c.) injection or intravenous (i.v.) infusion, the

antagonistic effects of Mo were alleviated. In addition, dietary Cu was not affected by abomasal injection of Mo, meaning that rumen was the primary site of antagonism. Some MoS_4 can be absorbed into the blood stream of ruminants where it complexes with circulating Cu by reacting with albumin. These Cu-MoS_4-albumin complexes may inhibit the formation of Cp in the liver (Suttle, 1991).

In the rumen, Fe reacts with sulfate to form ferrous sulfide (FeS). This interaction prevents rapid absorption of sulfide from the rumen. The trapped sulfide is thought to be released in the acidic environment of the abomasum, and to react thereafter with Cu to form insoluble and unabsorbable CuS (Bremner et al., 1987; Suttle et al. 1984).

Enzymes Associated with Copper

Many of the clinical symptoms of Cu deficiency are due to the reduction in function of Cu-dependant metalloenzymes, such as cytochrome C oxidase (CCO), copper/zinc superoxide dismutase (Cu/Zn SOD), dopamine β-hydroxylase, and tyrosinase.

Cytochrome C oxidase is the terminal enzyme in the electron transport chain, and is known to contain two to three Cu atoms essential for its function as a catalyst for oxidative phosphorylation (i.e. energy production). Studies have demonstrated that CCO activity was substantially reduced in Cu-deficient mice (Prohaska, 1983) and rats (Prohaska and Smith, 1982). Chao et al. (1994) reported a reduction in ATP synthase and CCO activity in heart tissue of Cu-deficient rats. In sheep, reduced CCO activity was associated with neonatal enzootic ataxia involving incomplete myelination of

nervous tissue in the spinal chord (Fell et al., 1965). As early as 1939, cattle with a condition called 'falling disease' were examined and diagnosed with cardiac lesions and extremely depressed blood and liver Cu concentrations (Bennets and Hall, 1939). These clinical symptoms were comparable to those reported by Chao et al. (1994) in Cu-deficient rats that had cardiac lesions and reduced CCO activity.

One of the most important roles of Cu is to function as a cofactor in antioxidant enzymes. The major antioxidant enzyme found in the cytoplasm of cells is Cu/Zn SOD. Its function is to mediate the reduction of two superoxide anions ($\bullet O_2^-$) to form one molecule of hydrogen peroxide (H_2O_2). This reaction is crucial to cellular viability, as the $\bullet O_2^-$ is deleterious to cell life. Production of reactive oxygen metabolites (ROM) occurs as a result of natural metabolic processes in the body. Organisms are said to be in a state of oxidative stress when production of ROM exceeds the activity of antioxidant mechanisms. Often, sickness or environmental stress can cause an increase in ROM production. Umeki et al. (1987) measured SOD and $\bullet O_2^-$ levels in immunocompromised human patients with and without pneumonia, and discovered that even though all compromised patients showed elevated $\bullet O_2^-$ levels and decreased SOD activity, those compromised patients with pneumonia showed significant increases in $\bullet O_2^-$ concentration with a concurrent decrease in SOD function. Copper/zinc SOD is a major regulator of $\bullet O_2^-$ levels, and derangement of any component of the enzyme (i.e. Cu deficiency) may reduce the control of ROM production (Miller and Brezinskia-Slebodzinska, 1993).

Copper is a component of the enzyme, dopamine β-hydroxylase, which is required for the production of epinephrine and norepinephrine (NE) from dopamine in the adrenal gland. Both substances are important for transmission of nerve impulses, mediating stress responsiveness, and indirectly stimulating metabolism. Prohaska and Cox (1983) found that Cu-adequate rats had higher NE concentrations than Cu-deficient rats, and that a Cu injection to the Cu-deficient rats reversed this effect. Norepinephrine activates 5' deiodinase, which converts thyroxine (T4) to triiodothyronine (T3). About 90% of thyroid hormone is secreted as T4, yet T3 is four times more potent in its biological activity. Compared to controls, Lukaski et al. (1995) found that deiodinase activity in liver and brown adipose tissue was decreased by 46 and 36%, respectively, in Cu-deficient rats. Newborn lambs born to ewes fed a low-Cu diet had lower rectal temperatures than lambs born to ewes fed a high-Cu diet (Carstens et al., 1999). Additionally, the lambs from the low-Cu ewes had lower T3:T4 ratio and NE turnover than lambs from the high-Cu ewes. The reduction in NE turnover and plasma T3:T4 ratios suggest impaired stimulation of brown adipose tissue (BAT) 5' deiodinase activity possibly due to a reduction of dopamine β-hydroxylase stimulated production of NE.

Copper is also a cofactor of the enzyme tyrosinase, which catalyzes the conversion of the amino acid, tyrosine, to melanin in hair and wool. Copper-deficient cattle often have a rough coat, loss of hair pigmentation over the withers, and 'copper spectacles' (dilute hair color around eyes) due to reduction in tyrosinase activity (Wikse et al., 1992). Liver Cu is the most reliable measure of overall Cu status, serum Cu

concentrations show marked decline only when liver Cu is depleted to a level below 30 ppm DM (Hardt et al., 1991).

Enzymes Associated with Zinc

Zinc was recognized as an essential nutrient for mammals in the early 1930s based on experiments in mice and rats (Todd et al., 1934), but was not believed to be an essential nutrient for farm animals until 1955 (Tucker and Salmon, 1955). Miller et al. (1966) reported that Zn deficiency reduced feed intake in ruminants, and that force-feeding a ration to Zn deficient animals was fatal. Zinc functions mostly in enzyme systems, so it was generally agreed that symptoms of a deficiency were manifest due to a reduction in the activity of some integral enzyme. In 1970, Miller suggested that the enzyme must be involved in a major biochemical pathway that prevented an animal to utilize energy, obtain a crucial compound, or that it blocked some reaction that caused accumulation of toxic products. Current research has shown that Zn is an essential element in key metalloenzymes such as carbonic anhydrase (CA), Cu/Zn SOD, and ribonucleic acid (RNA) polymerase.

Carbonic anhydrases are widespread in nature, being found in animals, plants, and certain bacteria. Carbonic anhydrase plays an important role in the regulation of rumen pH and volatile fatty acid (VFA) absorption. Aafjes (1967) reported that high concentrations of CA in the epithelium of ruminant forestomachs increased VFA absorption. He stated that CO_2 could be absorbed into the epithelial cells where it would combine with H_2O in the presence of CA producing HCO_3^-. The production of HCO_3^- in the rumen epithelium also produced a proton gradient favorable for VFA absorption

(Stevens, 1970). In fact, Wang et al. (1996) discovered that the concentration of mRNA that codes for the CA gene is five folds higher in mature lambs, which rely more heavily on rumination, compared to neonatal (pre-ruminant) lambs.

Though Cu/Zn SOD requires both Cu and Zn for proper function, its activity is less dependent upon Zn status. Rats that were fed Zn deficient diets displayed clinical signs of deficiency, but tissue Zn concentrations and Cu/Zn SOD activities were not decreased compared to rats fed Zn-adequate diets (Bray et al., 1986; Hammermueller et al., 1987; Taylor et al., 1988). There was, however, an increase in production of free radicals by lung microsomes in Zn-deficient rats. The actual mechanism by which Zn deficiency affects lung microsome free radical production is unknown; however, evidence suggests that zinc's role in structural integrity of tissue proteins is diminished during Zn deficiency (Hammermueller et al., 1987).

Ribonucleic acid polymerase, the enzyme responsible for the DNA-directed synthesis of RNA, contains two bound Zn atoms (Voet et al. 1999). The production of RNA during transcription mediates the translation of this nucleic acid into specific proteins. Flynn et al. (1984) suggested that Zn deficiency may have depressed production of key proteins responsible for proliferation, interfered with processing of antigens by accessory cells, or was involved in the loss of T-cell active state or function. James et al. (1987) found that T-cell proliferation was reduced in Zn deficient rats. This study also reported a 41% reduction in thymus weight in Zn-deficient rats compared to controls. This reduction may be attributed, in chronic deficiency cases (12 wks in rats), to a reduction in RNA processing. Though the exact mechanism by which these

proliferative responses were depressed by Zn deficiency is still unknown, each of the cellular activities listed above involves DNA replication, involving many enzymes including RNA polymerase.

Physiological Role of Selenium

Maintenance of adequate selenium status is important for control of oxidative metabolism (Voet et al., 1999) and in the prevention of nutritional muscular dystrophy, also known as white muscle disease (WMD). Selenium deficiency has also been shown to impair immune function in rodents (Mulhern et al., 1985) and calves (Swecker et al., 1988; Droke and Loerch, 1989; Stabel et al., 1990).

Glutathione Peroxidase. The main selenoprotein found in erythrocytes is glutathione peroxidase (GSH-Px); consequently, Se content is highly correlated with GSH-Px activity in whole blood (Miller et al., 1988). Glutathione peroxidase was discovered by Mills (1957), who reported that glutathione (GSH) in the presence of this enzyme helped protect erythrocytes from oxidative damage by H_2O_2. Rats with Se deficiency showed reduced GSH-Px activity compared to Se-adequate controls (Rotruck et al., 1972). This lead to the discovery that GSH-Px was actually a selenoenzyme (Rotruck et al, 1973) consisting of four subunits each associated with one Se atom (Ladenstein, 1979). Glutathione peroxidase functions by mediating the simultaneous reduction of H_2O_2 by oxidation of GSH. Hydrogen peroxides are optimally reduced by GSH-Px in the cytosol prior to their incorporation into unsaturated fatty acids; therefore, prevention of lipid damage is the primary role of this enzyme, however, there was little evidence supporting the assumption that GSH-Px could reduce membrane phospholipid

peroxides (hydroxides incorporated into unsaturated fatty acids) (McCay et al. 1981). Subsequently, researchers discovered the existence of phospholipid hydroperoxide glutathione peroxidase (PH-GPx), an enzyme with a function analogous to that of GSH-Px, with the obvious exception that its main substrate is phospholipid hydroperoxide (Weitzel et al., 1990). The study reported a rapid decrease in GSH-Px activity during Se depletion (undetectable level after 130 d), with a prolonged PH-GPx activity (30 to 70% of controls) occurring even after 250 d. Thus, a decrease in GSH-Px activity correlated to manifestation of clinical Se deficiency. Glutathione peroxidase has been shown to play in integral role in immune function as well as in the early stages of inflammation during wound repair (Munz et al., 1997).

Iodothyronine Deiodinase. The iodothyronine deiodinases are selenium-containing enzymes that catalyze the activation and inactivation of the thyroid hormones that regulate metabolic processes. The deiodinase family consists of three members that differ with regard to their tissue distribution and their specific role in deiodination of thyroxine (Behne and Kyriakopoulos, 2001). Located primarily in the thyroid, liver, kidney, and pituitary, type 1 deiodinase catalyzes monodeiodination of T4 at the 5' position of the phenolic ring producing T3 (active) or at the 5-position of the tyrosyl ring producing reverse T3 (inactive). Type 2 deiodinase is expressed mainly in the mitochondrial membranes of brain, brown adipose tissue, and placenta (Behne et al., 1990). It only catalyzes 5' deiodination serving as the local regulator of T3 production in tissues that contain this enzyme. Steers fed a selenium deficient diet (< 0.015 mg Se/kg) for 23 weeks showed a 62% increase in plasma T4 concentration with a concurrent 35%

decrease in plasma T3 concentration compared to controls (Arthur et al., 1988). The authors suggest that this was due to suppression of iodothyronine 5'-deiodinase activity in Se-deficient individuals.

Physiological Role of Vitamin E

Vitamin E is essential to such body functions as growth, reproduction, immunity, and tissue integrity (McDowell et al., 1996). In nature, there are several forms of vitamin E capable of activity, the most potent being α-tocopherol with β, γ and δ-tocopherols being 50, 20 and 10% as bioactive as the α form, respectively (Huber, 1988). Hoekstra (1975) reported that both vitamin E and GSH-Px are an integral part of the antioxidant system responsible for controlling tissue ROS and lipid peroxide levels. While GSH-Px functions in the cytosol of the cell, vitamin E is a highly active scavenger of ROS and lipid peroxides as a component of lipid membranes.

Alpha-tocopherol as an antioxidant. It is generally accepted that the primary metabolic function of α-tocopherol is to serve as an antioxidant, preventing free radical peroxide damage to cellular membranes (Huber, 1988). Reactive oxygen metabolites can be hazardous to biological membranes by initiating chain-reactions of polyunsaturated fatty acids (PUFA). Alpha-tocopherol serves to 'break' the chain-reaction by intercepting oxygen radicals, particularly superoxide radicals. It does this by reacting with the free radical carbon center of a PUFA resulting in the oxidation of the vitamin first to a semi-stable radical form and finally to α-tocopheryl quinone, the spent form of vitamin E which no longer acts as a radical scavenger (Halliwell and Gutteridge, 1985). The authors also suggest that there is a possible recycling of the semi-stable

tocopherol radical involving glutathione, glutathione peroxidase, and ascorbate (a form of vitamin C), resulting in the reproduction of α-tocopherol, however, the mechanism is still unclear at the moment. Alpha-tocopherol acts as a radical scavenger quenching radicals produced in the biological membranes enabling proper cell structure, and ultimately, proper cell function essential for healthy bodily function.

Non-specific Immunity

Non-specific immunity is the body's first line of defense against invading pathogens and involves those cells with the ability to non-specifically phagocytize foreign material. The primary leukocytes involved in this process are polymorphonuclear neutrophils (PMN) and mononuclear macrophages. The mechanism by which these phagocytic cells destroy foreign material is called the respiratory burst and involves exposing engulfed bacteria to ROS. The presence of Cu/Zn SOD and glutathione peroxidase in the cytosol facilitates the conversion of superoxide radical to less harmful metabolites.

Macrophages produce and release interleukin-1 (IL-1), interleukin-6 (IL-6), interleukin-12 (IL-12), and tumor necrosis factor-α (TNF-α), and are key components in the initiation and regulation of an immune response. Interleukin-1 activates T helper 2 (Th-2) cells primarily involved in the humoral immune response, acts on the brain to produce fever, and stimulates the liver to mobilize a pool of amino acids for the purpose of acute-phase protein production. Interleukin-6 acts in much the same way as IL-1 though is far less potent. Interleukin-12 stimulates T helper 1 (Th-1) cells to produce IL-

2 and interferon-α, as well as mimicking the cytotoxic activities of Th-1 cells aiding in the cellular immune response.

Excess ROS production can occur during incidences of pathogenic or parasitic infection, transit stress, or antioxidant deficiencies (Nockels, 1996). Immune cells are particularly sensitive to oxidative stress due to the high percentage of PUFA in their plasma membranes and the increased production of ROS, which is part of their normal activity (Knight, 2000). Copper and Zn deficiency in animals has been shown to reduce the function of phagocytic cells. This can be associated with the decreased availability of the enzyme Cu/Zn SOD required for the pathogen killing ability of neutrophils and macrophages (Knight, 2000).

Copper-deficient steers showed decreased Cu/Zn SOD activity as well as a fewer number of viable neutrophils when compared *in vitro* to controls (Boyne and Arthur, 1986). In this study, cattle were exposed to high concentrations of the Cu antagonists Mo and Fe, producing a secondary Cu deficiency. The Cu-deficient cattle showed a decrease in the number of viable neutrophils *in vitro*, as well as a decreased ability to kill *Candida albicans* when compared to animals on a control diet. In contrast, Genglebach et al. (1997) found no difference in ability of neutrophils from secondary Cu-deficient cattle to phacocytize *C. albicans*, and reduced pathogen killing ability by the neutrophils failed to reach significance (P = .15). Feeding diets low in Cu, Jones and Suttle (1981) produced a primary deficiency in ewes, and reported no decrease in neutrophil killing ability in Cu-deficient animals. Xin et al. (1991) reported that severe Cu deficiency in cattle (18 mg Cu/kg DM liver) had no effect on the phagocytic ability of neutrophils, but

decreased their microbicidal activity by 35%. This differs from Jones and Suttle (1981) and Arthington et al. (1995), who found no difference in phagocytic or microbicidal activity in marginally Cu-deficient ewes. These discrepancies may provide some evidence that severe vs. marginal deficiency, as well as primary vs. secondary deficiency may affect PMN activity in a highly integrated and variable manner.

Using cell crossover experiments in neonatal mice, James et al. (1987) determined that macrophages from Zn-deficient mice possessed reduced ability to stimulate T-cell proliferation. When macrophages from Zn-deficient mice were combined with control T-cells (from Zn- adequate mice), proliferative responses were lower compared to control macrophages combined with control T-cells. Researchers suggest that this resulted from a decrease in IL-12 secretion, which is responsible for inducing Th-1 cell proliferation when stimulated by macrophages. Eugui et al. (1994) cultured monocytes and found that pretreatment with antioxidants reduced the production of TNF-α and other cytokines induced by LPS challenge *in vitro*. Cytokines are considered stimulants of ROS production, and reducing their release would decrease production of harmful ROS. Webel et al. (1998) showed that plasma peak levels of IL-6 were reduced 35% for vitamin E treated pigs compared to non-supplemented pigs, thus showing for the first time *in vivo*, cytokine relationship to antioxidant treatment.

Humoral Immunity

Humoral immunity involves those lymphocytes that originate in the bone marrow then migrate to the secondary lymphoid tissue. These lymphocytes are called B-lymphocytes (B cells) and are involved in the production of a class of proteins called

immunoglobulins (Ig) that are produced to bind specifically to antigens. There are currently five classes of Ig including IgG, IgM, IgA, IgD, and IgE. In ruminants, IgG is the most abundant Ig in serum and is produced in large quantities when an animal is exposed to an antigen the second time, also known as a secondary immune response. The pentamer, IgM, is the second most abundant Ig in serum and is produced during both a primary and secondary immune response, though its presence is masked by the large increase in production of IgG during a secondary response. One of the purposes of the antibodies is opsonization of the invading pathogen. Opsonization is simply the binding of antibodies to their specific antigen on the invading pathogen causing it to be "tagged" for destruction by the non-specific immune cells.

The effects of trace minerals on rodent humoral immunity have been well documented. Perinatal copper deficiency in C58 mice during the first six postnatal weeks resulted in splenomegaly and thymal atrophy (Prohaska and Luckasewycz, 1983). Researchers suggest that decreased developement of the thymus in Cu-deficient mice could result in altered T cell subsets. In fact, Lukasewycz et al. (1985) determined that when a Cu-deficient splenomegaly was observed, there was a large increase in the number of B cells, while T cell counts remained equivalent to controls. Additionally, Prohaska and Luckasewycz (1989) found larger spleens and smaller thymus glands in Cu-deficient Swiss albino mice as well. They also reported a decrease in the number of IgM-secreting cells in response to sheep erythrocytes in animals with moderate to severe Cu deficiency. Flynn (1984) suggests that a zinc deficiency may alter lymphocyte responsiveness and active site functionality, therefore, depressing proliferative

responses. In fact, Zn tissue concentrations in the thymus and liver of mice decreased following sensitization of the humoral system with sheep erythrocytes (Verbanac et al., 1998). Lymphocyte membrane-specific functions, such as antigen recognition and binding, functioning receptors for cytokines and T cell adherence are affected by membrane fluidity and physical state, both of which are determined by the lipid acyl chain profile of the membrane. Importantly, these properties are affected by ROS-induced modification, and consequently, any mechanism in place to regulate ROS activity and production can improve the function of these cells (Knight, 2000). For this reason, Se and vitamin E are important, as they are directly responsible for control of lipid peroxidation in and around cellular membranes.

While trace mineral deprivation consistently impairs humoral immune function in rodents, evidence that this occurs in cattle is less consistent and highly variable. Ward and Spears (1999) reported that Cu deficiency alters immune response in seven month-old angus steers only after extended periods, and suggest that the immune system of cattle is not as sensitive to the adverse effects of supplemental Mo as are other physiological systems. In this study, 42 steer calves were determined to be copper deficient (0.55 ± 0.05 mg/L plasma Cu) prior to injection of 22 of these steers with 90 mg of Cu as copper glycinate 28 d prior to weaning. Treatments were assigned with factors being 0 or 5 mg Cu, as copper glycinate, per kg of DM and 0 or 5 mg Mo, as Na_2MoO_4, per kg of DM. The steers were kept in their prior Cu treatment groups respectively, and ship stressed by transporting 2.5 h and holding an additional 9.5 h on trailers. Cu status had no effect during the receiving (arrival of steers) phase, but Cu

supplementation altered immune function during growing phase variably. Copper supplementation increased humoral response to ovalbumin during the growing phase ($P < 0.10$), and increased antibody titers to porcine erythrocytes in cattle that were ship stressed, but decreased antibody titers to porcine erythrocytes of unstressed cattle ($P < 0.05$). Additionally, despite Mo supplementation depressing Cu status during the growing phase, it had no effect on humoral immune function. Branum (1999) reported no difference in antibody titers to a primary injection of KLH in calves whose dams were supplemented prenatally with 0, 20, 40 or 80 ppm Cu DM. However, 14 d after the secondary inoculation, calves born to 0-Cu cows had higher KLH antibody titers compared to calves from 80-Cu cows. A post-weaning diet of 0, 10 or 40 ppm Cu DM fed to the same calves showed that animals receiving 0 ppm Cu DM had lower IgG response to OVA than those fed 10 and 40 ppm Cu DM (Davis, 1999).

Stanton et al. (1998) reported that treatment with trace minerals did not significantly affect the humoral immune response to IBR and PI_3. Steers were divided into three groups receiving different trace mineral treatments (1) Control- low level of supplemental trace minerals; (2) Inorganic- supplemental trace minerals from zinc sulfate, manganese sulfate, copper sulfate and cobalt carbonate at isomineral levels to treatment 3; (3) Organic- supplemental trace minerals from zinc amino acid complex, manganese amino acid complex, copper amino acid complex, and cobalt glucoheptonate per steer per d. Despite the fact that supplementation with trace mineral either maintained or increased liver concentrations of each mineral, IBR and IP_3 titers were not different in any of the groups receiving various treatments. However, it did appear that

IBR titers were numerically, higher ($P > 0.05$) for the inorganic and organic treatments compared to controls.

Droke and Loerch (1989) reported that injection of Se and/or vitamin E does increase antibody titers. Steers injected with 25 mg Se (as Na_2SeO_3) plus 340 IU vitamin E had higher titers to *Pasteurella heamolytica* on d 7 and 14 than non-injected steers. In a subsequent trial, steers that were injected with 25 mg Se plus 340 IU vitamin E showed increased titers to *P. heamolytica* compared to those that received either no supplement or 340 IU vitamin E alone, however titers were not different from those supplemented with 25 mg of Se alone. Conversely, in a following trial, there was no difference in titers to *P. heamolytica* in steers receiving 25 mg Se plus 340 IU vitamin E as compared to those receiving no supplementation. Selenium alone or in combination with vitamin E improved immunocompetence over animals receiving no supplement or vitamin E alone as evidenced by large increases in IgG titers in response to *P. heamolytica* injection. However, the variability in the results obtained in each trial indicates that no firm conclusions can be drawn as to the effects of supplementation on the humoral response to a particular antigen.

Cellular Immunity

The cellular, or cell-mediated, immune response involves those lymphocytes that originate in stem cells of the bone marrow, and then migrate to mature within the thymus gland. They are named T cells based on their location of maturation and consist of Th-1, Th-2, and cytotoxic T (Tc) cells.

Lectins, a plant-based family of proteins, are often used as mitogens in order to study cellular immunity. By definition, a mitogen is a molecule that stimulates cell division. Most mitogens are specific for either B cells or T cells, though some nonspecific mitogens stimulate both cell types. Though the mitogens phytohemagglutinin (PHA) and concanavalin A (Con A) are primarily T cell stimulators, they often have a slight effect on B cells. Pokeweed mitogen (PWM) is a nonspecific mitogen with the ability to stimulate both types of cells and B cell-dependant antibody production. The simplest test of lymphocyte responsiveness is the *in vivo* skin-swelling test. In this test, the mitogen of choice is injected intradermally, and change in skin thickness is measured over time. The resultant skin swelling is similar to type IV hypersensitivity, though peak swelling occurs some period of time after injection. The delay in time needed for T cells to migrate to the injection site is the reason the reaction is called a delayed hypersensitivity reaction. Lymphocyte responsiveness can also be assessed *in vitro* by counting labeled lymphocytes produced after incubation. Purified T cells are cultured with an antigen or mitogen added for 48 to 96 hours. Tritium-labeled thymidine is added to the culture in order to assess the amount of proliferation. Lymphocytes that are not proliferating do not incorporate the labeled thymidine, but dividing lymphocytes incorporate the thymidine into newly synthesized DNA.

Effects of mineral deficiencies on cellular immune response to mitogens have been widely studied in non-ruminants using both *in vivo* and *in vitro* techniques. Copper deficiency in rats and mice has been shown to reduce the number of Th cells and Tc cells (Bala et al., 1991; Mulhern and Koller, 1988; Lukasewycz et al., 1985). Furthermore,

there was also a reduction in proliferative response to mitogens during Cu deficiency (Bala and Failla, 1992; Bala et al., 1991; Lukasewycz et al., 1985). Bala and Failla (1992) suggest that reduced responsiveness is due to the inability of Th-1 cells to produce adequate IL-2 during Cu deficiency, and reported that Cu repletion restored IL-2 production after one week. James et al. (1987) reported that long-term Zn deficiency (< 0.2 μg/g dietary Zn for 12 wks) reduced the ability of T-cells to proliferate when stimulated by control macrophages in cell crossover experiments. The authors state that the competence of the peripheral T cells would be affected when the half-life of the average T cell is exceeded by the duration of deficiency and thus the pool of competent cells becomes depleted. The ability of Zn-deficient macrophages to stimulate T cell proliferation, not a decrease in T cell function, was observed due to a short-term Zn deficiency (6 wks).

Direct relationships between trace mineral status and cellular immune function in ruminants have been less evident than in non-ruminant models. Ward and Spears (1999) reported that dietary Cu treatment and transit stress had minimal effect on delayed type hypersensitivity (DTH) reaction to dinitrochlorobenzene (DNCB) with Cu supplementation lowering DTH in unstressed steers, and increasing DTH in stressed steers. Branum (1999) reported that skin-swelling response to intradermal PHA was higher for calves born to cows fed 80 ppm Cu DM, compared to calves born to cows fed 0, 20, and 40 ppm Cu DM. Skin-swelling response to KLH was not different among any treatments, but those calves born to cows consuming the 40- and 80-Cu diets showed a greater decline in swelling from the maximum (12 h post injection) to the 48 h

measurement compared to calves from 0- and 20-Cu cows. Davis (1999) reported that calves from cows receiving 0-, 20-, and 40-Cu and subsequently fed 40 ppm Cu DM had the highest numerical skin-swelling response and did not statistically differ from each other. Calves from cows with 0-Cu and fed 0-Cu postweaning had the lowest skin-swelling responses.

Stanton et al. (1998) reported that skin-swelling response to PHA injection in cattle was not different between inorganic and organic trace mineral treatments through 24 hours post injection. However, in the group receiving the high level of inorganic mineral treatment, there was greater skin thickness at 48 h post injection compared to controls. The authors stated that it appeared that the high levels of inorganic trace mineral stimulated cellular immune response compared to controls. This is in contrast to what the authors subsequently report that no difference in skin swelling response to PHA inoculation among steers recieving high levels of organic or inorganic trace minerals was observed compared to controls (Stanton et al., 2000).

Stabel et al. (1990) added sodium selenite (Na_2SeO_3) (from 0 to 3200 ng • mL^{-1}) to bovine lymphocytes after they were incubated with Con-A, PHA, or PWM. Optimal enhancement of stimulation to PWM (T- and B cell mitogen) occurred with the addition of 50 to 400 ng Na_2SeO_3 • mL^{-1} to the culture medium while the T cell mitogens (Con A and PHA) were minimally affected by Se repletion. Researchers suggested that the enhancement of proliferation in PWM cultures resulted from increased production of cytokines such as IL-1 and IL-2 by Th cells.

CHAPTER III

NUTRITIONAL MODULATION OF IMMUNITY AND PHYSIOLOGICAL RESPONSES IN BEEF CALVES

Introduction

Feedlots are often located great distances from ranches of beef cattle producers, which means that cattle will need to be transported at some point in time during growth and development. During these long journeys adverse weather conditions, fasting, crowding and contact with other infected animals places greater economic importance on immune function of these cattle. It is important to understand the effect that transit stress has on steers after they leave the ranch. Transporting steers causes oxidative stress that can cause cellular and tissue damage. This cellular and tissue damage can be detrimental to immune function by disrupting the control of expression of various cytokines, and inhibiting the ability to produce functional lymphocytes and antibodies (Knight, 2000). In order to combat the detrimental effects of oxidative stress, it is important that cattle maintain adequate levels of antioxidants. The trace minerals Cu, Zn, Se and vitamins A and E act as antioxidants and as cofactors for important enzymes with antioxidant activity.

Research Objectives

The primary objective of this experiment was to determine if injections of the supplemental the trace minerals Cu, Zn, Mn, Se and vitamin E prior to transit stress would affect humoral and cellular immunity in growing calves. The secondary objective

of this experiment was to determine the trace mineral and vitamin E states of calves in response to injections with Multimin™ and vitamin E.

Materials and Methods

Experimental Design

Twenty-four crossbred steer calves were weaned, adapted to a growing diet, and trained to eat from Calan electronic gate feeders for 28 d. Steers were assigned to pens equipped with Calan gate feeders (6 steers per pen) located at the Nutrition Physiology Center (NPC), Texas A&M University, College Station, Texas. This facility contained 6.1 x 12.2 m pens with a cement floor and a roof that covered approximately half of the pen area. The diet (Table 1) was formulated to meet protein and energy requirements for steers to gain 0.7 kg/d (NRC, 2000). Supplemental Cu, Zn, Se and vitamin E were not added to the diet, but 0.3% S and 900 ppm Fe (DM Basis) were added to partially impair copper absorption.

Steers were blocked by BW and randomly assigned to one of four treatments in a 2 x 2 factorial arrangement as follows: (1) no transit stress and a saline injection, (2) no transit stress and a trace mineral and vitamin E (TM/VitE) injection, (3) transit stress and a saline injection and (4) transit stress and a TM/VitE injection. The timeline for TM/VitE injections in cattle is shown in Figure 1 and includes immune function experiments. The TM/VitE supplement was administered on d 0 and 21 of the experiment and consisted of s.c. Multimin™ injections at a dose of 0.03 mL/kg of BW to provide 0.3, 0.6, 0.15 and 0.6 mg of Cu, Mn, Se, and Zn/kg of BW, respectively, and s.c. injections of vitamin E (Vita E™) at 0.03 mL/kg BW in order to provide 9 IU of vitamin

Table 1. The ingredient and nutrient composition of the experimental diet.

Ingredient	**Amount, % (as fed)**
Cottonseed hulls	45.91
Alfalfa hay	10.0
Cottonseed meal	18.0
Corn, whole	16.0
Molasses	8.0
Calcium sulfate	1.25
Salt	0.5
Iron sulfate	0.34
Nutrient*	**Amount, % (DM basis)**
NE_m Mcal/kg	1.52
NE_g Mcal/kg	0.92
CP, %	18.7
ADF, %	30.9
K, %	1.67
Ca, %	0.94
S, %	0.57
P, %	0.53
Fe, ppm	1540.0
Zn, ppm	50.0
Cu, ppm	19.0
Mn, ppm	36.0
Mo, ppm	1.7
Se, ppm	0.32

*Nutrient analysis obtained from DHIA Forage Testing Laboratory, Ithaca, NY

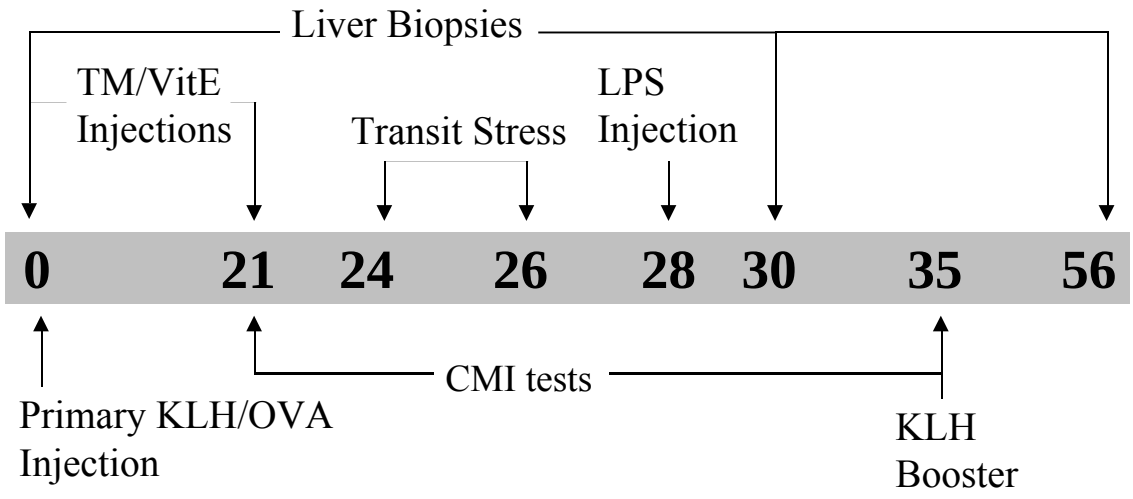

Figure 1. Experimental timeline. TM/VitE steers were injected with Multimin^{TM} to provide 0.3, 0.6, 0.15 and 0.6 mg of Cu, Mn, Se, and Zn and 9 IU of vitamin E/kg of BW on d 0 and 21 of the experiment.

E/kg of BW. Those calves not receiving trace mineral supplement were injected s.c. with an equal volume of saline.

On d 24 of the experiment, the transit-stressed steers were transported 813 km to the Amarillo Research and Extension Center and held overnight with access to water only. The following day steers were transported to the Uvalde Research and Extension Center (777 km) where they received access to hay and water overnight before being transported 408 km back to College Station. The non-transit-stressed steers remained at the NPC in College Station. During the three-d transit-stress period, the non-transported calves that remained in College Station were exposed to harsh weather conditions including heavy rain, strong winds and a 20 °C decrease in air temperature.

The day after return of the transit-stressed cattle (d 27)**,** all steers were fitted with a jugular cannula. The following day (d 28) steers were administered lipopolysaccharide (LPS) from *Escherichia coli* O55:B5 (Sigma, St. Louis, Missouri) at 0.2 μg/kg of BW with 5 mL of buffered saline according to Elsasser et al. (1996). Rectal temperatures were measured at 0, 0.5, 1, 2, 4, 6, 10, 12, and 48 h during LPS challenge. On d 28 steer # 123 died 8 h after inoculation with LPS and the data for this animal was removed from further analysis.

Liver Mineral Status

Liver biopsies were performed on d 0 and 56 as well as 48 h after LPS challenge (d 30) using a biopsy probe (Sontec Instruments, Englewood, California). After a 25 cm^2 area was clipped with a #40 surgical blade and surgical prep was performed, 5 mL of lidocaine hydrochloride (VEDCO, St. Joseph, Missouri) was injected s.c. as a local

anesthetic. An incision was made in the 11th intercostal space, and the biopsy probe was inserted through the peritoneum and into the liver to obtain a sample (~0.2 g). Liver samples were then stored at -20° C until they were freeze-dried, digested and analyzed for liver mineral content using flame atomic absorption spectrophotometry (Thermo-Jarrel Ash model S11, 1990).

Serum Mineral Status

Blood samples were obtained via jugular venipuncture on d 0, 24, 26, 30, and 56, centrifuged, serum harvested, and stored at -20° C until samples were analyzed. Copper and Zn determination was preformed by diluting two mL of serum with an equal volume of phosphate buffered saline (PBS). Serum Cu and Zn concentration was then determined using flame atomic absorption spectrophotometry (Thermo-Jarrel Ash model S11, 1990) and results reported in mg/L. Blood was harvested via jugular venipuncture into evacuated tubes at 0, 24, 26, 30, and 56 d, and sent to Veterinary Medical Diagnostic Laboratory (TVMDL) Texas A&M University for analysis of whole blood Se concentration using atomic absorption spectrophotometry equipped with a hydride generator.

Serum Vitamins

Blood samples were obtained via jugular venipuncture on d 0, 24, 26, 35, and 56, centrgifued, serum harvested, and stored at -20 °C. Serum samples were analyzed for α-tocopherol, γ-tocopherol, and vitamin A concentrations by Central Analytical Laboratory, University of Arkansas (Fayettville, Arkansas), and reported in μg/mL.

Humoral Immunity

A diluted keyhole limpet hemocyanin (KLH) (Calbiochem, San Diego, California) and ovalbumin (OVA) (Sigma) inoculum was prepared by diluting one mg KLH and four mg of OVA with two mL of PBS in saponin. Steers were administered two mL of this KLH/OVA inoculum i.m. on d 0 and a booster injection on d 35. Blood samples were taken via jugular venipuncture into evacuated tubes on d 0 prior to initial KLH injection and on d 14, 21, 35, 42, 49, and 56. Serum was harvested and stored at -20 °C until ELISA procedures were used to determine the antibody titer to KLH. ELISA was performed by coating 96-well flat-bottom plates with 500 ng KLH/100 μL of 0.06M carbonate buffer (pH 9.6) per well and incubating at room temperature for 24 h. The plates were then washed with 0.01M PBS (pH 7.4) containing 0.5% Tween 20 three times prior to blocking with a 1% casein in PBS-Tween solution. The plates were washed again using the same procedure and stored at -20 °C until needed. Serum samples were double diluted from 1:400 to 1:6400 in PBS. Positive and negative controls and duplicate samples were added at 100 μL per well to the KLH coated plates and allowed to incubate at room temperature for 2 h. The washing procedure was then repeated three times and 100 μL/ well of horseradish peroxidase conjugated rabbit anti-bovine IgG (Sigma, St. Louis, Missouri) diluted at 1:30,000 with PBS was added and the plate allowed to incubate at room temperature for 1 h. The plates were washed three times and 100 μL of ABTS was added to each well and allowed to stand at room temperature for 10 min before measuring absorbance at 405 nm in a microplate reader.

Percent positivity was calculated for each sample by dividing the unknown sample absorbance by the absorbance of the positive control and multiplying by 100.

Cellular Immunity

A KLH and OVA inoculum was administered on d 0 and 35 (see *Humoral Immunity*). Cellular Immunity was assessed by measuring skin reaction on d 21, 35, and 56 to intradermal injections of 0.1 mL of 1.5 mg phytohemagglutinin (PHA) (Sigma) per mL of PBS and 0.1 mL of a 0.5 mg OVA per mL of PBS i.d. at one site per mitogen just posterior to the scapula. Injection sites were prepared by clipping a square area with a #40 surgical clipper blade and measuring initial skinfold thickness using microcalipers. Each site, scrubbed with betadine followed by 70% isopropyl alcohol, was then injected with its respective mitogen. Skin swelling responses to the injection were measured at 4, 8, 12, and 24 h with the microcalipers. The data is reported as an increase of skin-fold thickness (mm) in relation to skin thickness prior to injection.

Lymphocyte Proliferation

Blood samples were obtained via jugular venipuncture on d 0, 21, 28, 35, and 56 into 10-mL evacuated tubes containing heparin. Samples were stored at -4 °C until peripheral blood mononuclear (PBMC) cells were obtained by gradient centrifugation using Histopaque-1077. Isolated PBMC were washed twice in heparin buffered saline solution (HBSS) and resuspended in RPMI complete (RPMI supplemented with 20 IU/mL penicillin, 100 μg/mL streptomycin, 2.5 μg/mL amphotericin B, 10% FCS, 2 mM L-glutamine, and 0.1 mM b-ME). PBMC (100 ul; 1 x 10^6 viable cells per mL) were added in triplicate to 96-well round-bottom tissue culture plates containing 100 μL of the

RPMI complete. Ten μL of diluted KLH was added to each well in order to stimulate lymphocyte proliferation. Unstimulated wells were included as controls. The final volume of each well was 210 μL. Plates were incubated in 5% CO_2 at 37° C for 5 d before addition of 1 μCi of ^{3}H-methylthymidine to each well. After a further 16 h incubation, cells were harvested and the amount of thymidine incorporation (counts per minute, c.p.m.) was measured by liquid scintillation counting. The same procedure was used to determine lymphocyte proliferation to PHA and OVA. The data was reported as stimulation index (S.I.) calculated by dividing the sample cell counts with the negative control cell counts.

Statistical Analysis

Data from liver Cu and Zn, serum Cu and Zn, whole blood Se, vitamin E, vitamin A, humoral immunity, cellular immunity, lymphocyte proliferation, and rectal temperature were analyzed using the mixed model with repeated measures procedure of SAS (2000). The model included main effects of TM/VitE treatment, transit-stress treatment and day. There were no TM/VitE treatment x transit-stress treatment interactions, therefore data for the main effects of TM/VitE treatment and transit-stress treatment were presented separately. Weight gain, intake, growth rate, and feed conversion data were analyzed using general linear model procedure of SAS (2000) with TM/VitE treatment and transit-stress treatment as main effects. Values for parameters are reported as least square means and compared by least squared differences between main effects, and all differences with $P < 0.05$ are considered significant.

Results

Calf Performance Data

Steers that received TM/VitE injections grew slower ($P < 0.01$), had lower ($P = 0.10$) dry matter intake (DMI) and had lower ($P < 0.01$) feed efficiencies than saline-injected steers during the first 24 d of the experiment (Table 2). During the post-transit period (d 31 to d 56) DMI and ADG tended ($P = 0.10$) to be lower in TM/VitE-injected steers compared to saline-injected steers, although feed efficiencies were similar. At the beginning of the transit period (d 24), TM/VitE-injected steers had lower ($P < 0.05$) BW than saline-injected steers, however, upon completion of transit on d 26, the TM/VitE-injected steers tended ($P = 0.07$) to have retained a greater percentage of their initial BW compared to saline-injected steers (Table 3). There were no TM/VitE-treatment differences in BW changes during the LPS challenge or over the combined transit stress/LPS period (d 24 to d 31).

There were no differences in BW, ADG, or feed efficiency for transit-or non-transit-stressed steers throughout the entire experiment. However, during the post-transit period (d 31 to d 56), non-transit stressed steers tended to consume more ($P = 0.10$) DM than transit-stressed steers (Table 4). Upon return from transit (d 26), stressed steers had lower BW ($P < 0.05$) and had lost a greater percentage ($P < 0.001$) of their BW (8.2% loss) compared to non-transported animals who actually gained (1.7% gain) BW (Table 5). During the period between d 26 and d 31, non-transit-stressed steers tended to lose a greater ($P = 0.07$) percentage of total BW when compared to transit-stressed steers.

Table 2. The effects of trace mineral (TM) and vitamin E (VitE) injections on performance and feed efficiency in growing steers prior to and following transit.

Item	Saline-injected	TM/VitE-injected[†]	SE	P-value
Body weight, kg				
Day 0	235.8	234.1	6.0	0.84
Day 24	271.0	249.2	7.4	0.04
Day 31	250.0	235.8	7.9	0.20
Day 56	290.1	269.3	8.8	0.09
ADG, kg/d				
Day 0 - Day 24	1.46	0.625	0.18	0.003
Day 31 - Day 56	1.54	1.28	0.11	0.10
Daily intake, kg DM/d				
Day 0 - Day 24	7.90	7.25	0.25	0.06
Day 31 - Day 56	8.98	8.01	0.41	0.10
Feed efficiency, g gain/kg DM				
Day 0 - Day 24	182	86.5	0.02	0.006
Day 31 - Day 56	174	163	0.01	0.58

[†]TM and VitE injections were administered on d 0 and 21 of the experiment.

Table 3. The effects of trace mineral (TM) and vitamin E (VitE) injections on body weight during transit stress and LPS challenge.

Item	Saline-injected	TM/VitE-injected[†]	SE	P-value
Body weight, kg				
Day 24	271.0	249.2	7.4	0.04
Day 26	257.8	244.7	6.7	0.16
Day 31	250.0	235.8	7.9	0.20
Change in BW, %				
Day 24 - Day 26	-4.7	-1.7	1.2	0.07
Day 26 - Day 31	-2.8	-3.6	1.5	0.69
Day 24 - Day 31	-7.6	-5.4	1.4	0.23

[†]TM and VitE injections were administered on d 0 and 21 of the experiment.

Table 4. The effects of transit-stress treatment on performance and feed efficiency in growing steers prior to and following transit.

Item	Non-transit stress	Transit-stress[†]	SE	P-value
Body weight, kg				
Day 0	235.7	234.2	7.1	0.85
Day 24	259.3	260.7	7.1	0.89
Day 31	250.1	235.7	7.6	0.56
Day 56	287.2	272.2	8.2	0.22
ADG, kg/d				
Day 0 - Day 24	0.983	1.10	0.18	0.64
Day 31 - Day 56	1.43	1.39	0.11	0.82
Daily intake, kg DM/d				
Day 0 - Day 24	7.33	7.81	0.24	0.16
Day 31 - Day 56	8.97	8.02	0.40	0.10
Feed efficiency, g gain/kg DM				
Day 0 - Day 24	130	139	0.02	0.77
Day 31 - Day 56	159	177	0.01	0.35

[†]Steers were transported 2000 km from d 24 to d 26 and challenged with lippopolysaccharide between d 28 and d 31.

Table 5. The effects of transit-stress treatment on body weight during transit stress and LPS challenge.

Item	Non-transit stress	Transit-stress[†]	SE	P-value
Body weight, kg				
Day 24	259.3	260.7	7.1	0.89
Day 26	263.3	239.2	6.5	0.01
Day 31	250.1	235.7	7.7	0.19
Change in BW, %				
Day 24 - Day 26	1.7	-8.2	1.1	0.0001
Day 26 - Day 31	-5.1	-1.4	1.4	0.07
Day 24 - Day 31	-3.5	-9.5	1.3	0.004

[†]Steers were transported 2000 km from d 24 to d 26 and challenged with lippopolysaccharide between d 28 and d 31.

During the LPS challenge, non-transit-stressed steers tended ($P < 0.10$) to lose a greater percentage of their BW compared to transit-stressed steers. Overall, during the transit-stress/LPS period, the transported steers lost a greater percentage ($P < 0.01$) of BW compared to non-transported steers.

Liver Mineral Status

There was a TM/VitE treatment ($P < 0.001$), and TM/VitE treatment x day ($P < 0.001$) interaction for liver Cu. Liver Cu concentrations on d 0 of the study were similar for saline- and TM/VitE-injected steers and averaged 82.9 mg/kg DM. Liver Cu concentrations were 135 and 188% higher in TM/VitE-injected steers compared to saline-injected steers on d 30 and 56, respectively (Table 6; Figure 2). There was a TM/VitE treatment x day interaction for liver Zn. On d 0 liver Zn concentrations were similar for saline- and TM/VitE-injected steers and averaged 128 mg/kg DM (Table 6; Figure 2). On d 30 of the study, TM/VitE-injected steers had 21% higher ($P < 0.05$) Zn concentrations than saline-injected steers. However, by the end of the study, there were no treatment differences in liver Zn concentrations.

There was a transit-stress treatment x day interaction for liver Cu. On d 0 and d 30 liver Cu concentrations were similar for non-transit- and transit-stressed steers and averaged 84 and 98 mg/kg DM, respectively (Table 7; Figure 3). On d 56 of the study, transit-stressed steers had 30% higher ($P < 0.05$) Cu concentrations than non-transit-stressed steers. Transit-stress treatment, but not the transit-stress treatment x day interaction was significant for liver Zn. Over the entire study, transit-stressed calves had 12% higher ($P < 0.05$) liver Zn than non-transit-stressed calves (Table 7; Figure 3).

Table 6. The effects of trace mineral (TM) and vitamin E (VitE) injections on liver copper (Cu) and zinc (Zn) concentrations in growing steers.

Item	Day 0	Day 30	Day 56
Cu, mg/kg DM			
Saline	79.15[a]	58.31[a]	29.47[a]
TM/VitE[†]	89.03[a]	137.01[b]	84.89[b]
SE	8.20	6.45	4.20
Zn, mg/kg DM			
Saline	135.92[a]	134.14[a]	104.23[a]
TM/VitE	120.13[a]	161.92[b]	118.75[a]
SE	7.47	6.68	9.46

[ab]Values for each mineral within a given day with different superscripts differ ($P < 0.05$).

[†]TM and VitE injections were administered on d 0 and 21 of the experiment.

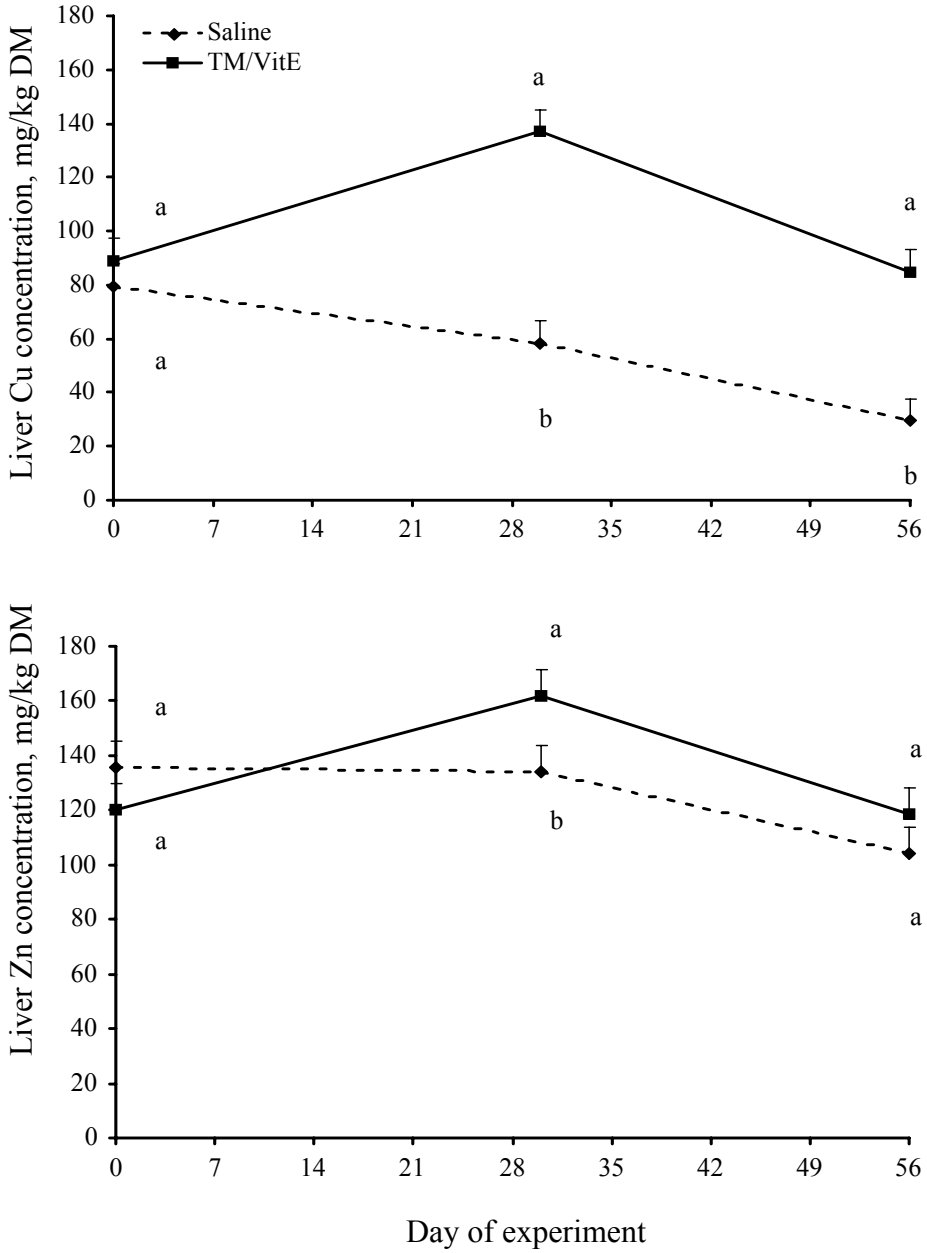

Figure 2. Effects of TM/VitE injections on liver Cu and Zn concentrations in growing steers. TM/VitE effect on liver Cu ($P < 0.001$); Day ($P < 0.001$); TM/VitE x day interaction ($P < 0.001$). TM/VitE effect on liver Zn ($P = 0.13$); Day ($P < 0.001$); TM/VitE x day interaction ($P < 0.01$); there were no significant TM/VitE x transit stress interactions for liver Cu or liver Zn. [ab]Values for each mineral within a given day with different superscripts differ ($P < 0.05$).

Table 7. The effects of transit-stress treatment on liver copper (Cu) and zinc (Zn) concentrations in growing steers.

Item	Day 0	Day 30	Day 56
Cu, mg/kg DM			
Non-Transit-stressed	81.18[a]	92.49[a]	49.70[a]
Transit-stressed[†]	87.33[a]	102.83[a]	64.63[b]
SE	7.88	6.16	4.01
Zn, mg/kg DM			
Non-Transit-stressed	120.82	139.40	105.58
Transit-stressed	135.23	156.68	117.41
SE	7.19	6.38	9.03

[ab]Values for each mineral within a given day with different superscripts differ ($P < 0.05$).

[†]Steers were transported 2500 km from d 24 to d 26 and challenged with lippopolysaccharide between d 28 and d 31.

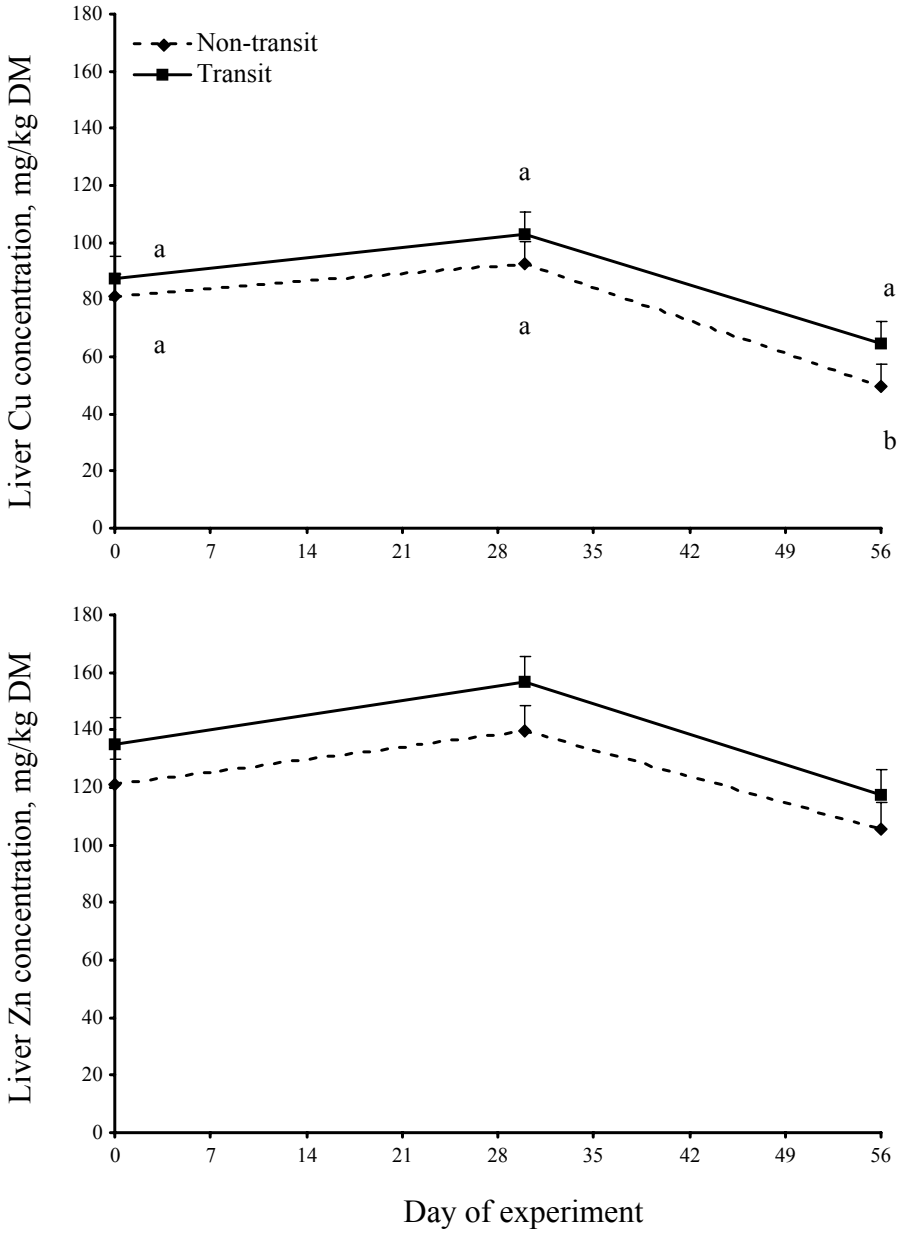

Figure 3. Effects of transit-stress treatment on liver Cu and Zn concentrations in growing steers. Transit stress effect on liver Cu ($P = 0.15$); Day $P < 0.001$); Transit stress x day interaction ($P = 0.06$). Transit stress effect on liver Zn ($P = 0.02$); Day ($P < 0.001$); Transit stress x day interaction ($P = 0.93$); there were no significant TM/VitE x transit stress interactions for liver Cu or liver Zn. ^{ab}Values for each mineral within a given day with different superscripts differ ($P < 0.05$).

Serum Minerals

The effects of TM/VitE treatment on serum Cu, Zn and whole blood Se are summarized in Table 8. There was a TM/VitE treatment ($P < 0.01$), and a TM/VitE treatment x day ($P < 0.001$) interaction for serum Cu. Serum Cu concentrations on d 0 of the study were similar for saline- and TM/VitE-injected steers and averaged 1.25 mg/L. Serum Cu concentrations were 14 and 23% higher ($P < 0.05$) in TM/VitE-injected steers compared to saline-injected steers on d 24 and 26, respectively. However, on d 30 and 56, there were no treatment differences in serum Cu concentration. There was a TM/VitE treatment x day interaction ($P < 0.01$) for serum Zn. Serum Zn concentrations were similar for saline- and TM/VitE-injected calves on d 0 and averaged 1.37 mg/L. On d 24 of the study, saline-injected steers had 37% higher ($P < 0.05$) serum Zn concentrations than TM/VitE-injected steers. However, on d 26, 30, and 56, there were no treatment differences in serum Zn. The TM/VitE treatment had no effect on whole blood Se concentrations.

The effects of transit-stress treatment on serum Cu, Zn and whole blood Se concentrations are summarized in Table 9. There was a transit-stress treatment x day interaction ($P < 0.001$) for serum Cu. Due to random assignment of treatments, non-transit-stressed calves had higher serum Cu concentrations on d 0. The effects of transit-stress treatment on serum Cu levels were evident on d 26 (return from transit stress) when transit-stressed steers had mobilized 18% more ($P < 0.05$) Cu than non-transit-stressed steers. Transit stress treatment did not affect serum Zn or whole blood Se concentrations, and there were no transit-stress treatment x day interactions observed.

Table 8. The effects of trace mineral (TM) and vitamin E (VitE) injections on serum copper (Cu), zinc (Zn) and whole blood selenium (Se) concentrations in growing steers.

Item	Day 0	Day 24	Day 26	Day 30	Day 56
Cu, mg/L					
Saline	1.32[a]	0.81[a]	0.87[a]	0.86[a]	0.56[a]
TM/VitE[†]	1.19[a]	0.92[b]	1.07[b]	0.99[a]	0.66[a]
SE	0.05	0.03	0.04	0.80	0.03
Zn, mg/L					
Saline	1.36[a]	1.08[a]	1.26[a]	0.89[a]	1.05[a]
TM/VitE	1.38[a]	0.79[b]	1.21[a]	0.90[a]	1.02[a]
SE	0.05	0.05	0.05	0.05	0.04
Se, µg/L					
Saline	109.91	209.56	230.16	216.36	228.98
TM/VitE	98.65	210.44	174.94	209.39	182.33
SE	7.62	26.34	27.85	21.88	16.82

[ab]Values for each mineral within a given day with different superscripts differ ($P < 0.05$).

[†]TM and VitE injections were administered on d 0 and 21 of the experiment.

Table 9. The effects of transit-stress treatment on serum copper (Cu), zinc (Zn) and whole blood selenium (Se) concentrations in growing steers.

Item	Day 0	Day 24	Day 26	Day 30	Day 56
Cu, mg/L					
Non-Transit-stressed	1.18[a]	0.86[a]	0.89[a]	0.88[a]	0.58[a]
Transit-stressed[†]	1.33[b]	0.87[a]	1.05[b]	0.97[a]	0.66[a]
SE	0.05	0.03	0.03	0.07	0.03
Zn, mg/L					
Non-Transit-stressed	1.36	0.94	1.18	0.92	1.01
Transit-stressed	1.37	0.93	1.09	0.88	1.06
SE	0.05	0.05	0.05	0.05	0.04
Se, µg/L					
Non-Transit-stressed	93.48	199.72	203.48	235.15	182.95
Transit-stressed	115.08	220.28	201.61	190.34	228.36[a]
SE	7.33	24.29	26.61	20.90	16.07

[ab]Values for each mineral within a given day with different superscripts differ ($P < 0.05$)

[†]Steers were transported 2000 km from d 24 to d 26 and challenged with lippopolysaccharide between d 28 and d 31.

Serum Vitamins

There was a TM/VitE treatment x day interaction ($P < 0.001$), for serum α-tocopherol concentration. On d 0 the mean α-tocopherol concentration was similar for saline- and TM/VitE-injected steers and averaged 2.5 μg/mL (Table 10). On d 24, and 26, the TM/VitE-injected calves had 173 and 73% higher ($P < 0.05$) serum α-tocopherol concentrations than saline-injected steers, respectively. The TM/VitE-injected steers had serum α-tocopherol concentrations that averaged 24% higher ($P < 0.05$) than saline-injected steers through d 56. There was a TM/VitE treatment x day interaction ($P < 0.01$) for serum γ-tocopherol concentration. Saline-injected steers had higher ($P < 0.05$) serum γ-tocopherol concentrations than TM/VitE-injected calves on d 24 and d 26 (Table 10). A TM/VitE treatment x day ($P < 0.001$) interaction was observed for serum vitamin A. TM/VitE-injected steers had 35% lower ($P < 0.05$) serum vitamin A concentrations compared to saline-injected steers on d 26 of the study (Table 10).

The effects of transit-stress treatment on serum α-tocopherol, γ-tocopherol and vitamin A concentrations are shown in Table 11. Over the entire study, transit-stressed steers tended to have lower ($P < 0.10$) serum α-tocopherol concentrations than non-transit-stressed steers (3.47 μg/mL vs 3.82 μg/mL). Transit-stress treatment ($P < 0.05$) affected serum γ-tocopherol concentrations, and a transit-stress treatment x day ($P < 0.001$) interaction was observed. Non-transit-stressed calves had higher ($P < 0.05$) serum γ-tocopherol concentrations than transit-stressed calves. There was a transit-stress treatment x day interaction ($P < 0.01$) for serum vitamin A concentration. Non-transit-

Table 10. The effects of trace mineral (TM) and vitamin E (VitE) injections on serum α-tocopherol, γ-tocopherol, and vitamin A concentrations in growing steers.

Item	Day 0	Day 24	Day 26	Day 35	Day 56
α-tocopherol, μg/mL					
Saline	2.82[a]	2.82[a]	3.05[a]	2.64[a]	2.60[a]
TM/VitE[†]	2.30[a]	7.69[b]	5.38[b]	3.27[b]	3.26[b]
SE	0.19	0.32	0.24	0.15	0.18
γ-tocopherol, μg/mL					
Saline	0.09[a]	0.14[a]	0.10[a]	0.14[a]	0.17[a]
TM/VitE	0.11[a]	0.05[b]	0.04[b]	0.11[a]	0.16[a]
SE	0.01	0.01	0.01	0.01	0.02
vitamin A, μg/mL					
Saline	0.21[a]	0.21[a]	0.20[a]	0.21[a]	0.22[a]
TM/VitE	0.23[a]	0.16[a]	0.13[b]	0.19[a]	0.20[a]
SE	0.02	0.04	0.01	0.02	0.01

[ab]Values for each vitamin within a given day with different superscripts differ ($P < 0.05$)

[*]TM and VitE injections were administered on d 0 and 21 of the experiment.

Table 11. The effects of transit-stress treatment on serum α-tocopherol (α-Vit E), γ-tocopherol (γ-Vit E), and vitamin A.

Item	Day 0	Day 24	Day 26	Day 35	Day 56
α-tocopherol, μg/mL					
Non-Transit-stressed	2.97[a]	5.61[a]	4.20[a]	3.14[a]	3.18[a]
Transit-stressed[†]	2.78[a]	4.89[a]	4.23[a]	2.76[a]	2.67[b]
SE	0.18	0.30	0.23	0.15	0.17
γ-tocopherol, μg/mL					
Non-Transit-stressed	0.10[a]	0.10[a]	0.11[a]	0.14[a]	0.18[a]
Transit-stressed	0.09[a]	0.09[a]	0.03[b]	0.11[b]	0.16[a]
SE	0.01	0.01	0.01	0.01	0.02
Vitamin A, μg/mL					
Non-Transit-stressed	0.23[a]	0.22[a]	0.18[a]	0.21[a]	0.20[a]
Transit-stressed	0.22[a]	0.15[a]	0.15[b]	0.18[a]	0.22[a]
SE	0.01	0.04	0.01	0.02	0.01

[ab]Values for each vitamin within a given day with different superscripts differ ($P < 0.05$)

[†]Steers were transported 2000 km from d 24 to d 26 and challenged with lippopolysaccharide between d 28 and d 31.

stressed steers had 20% higher ($P < 0.05$) serum vitamin A concentrations on d 26 compared to transit-stressed steers.

Immune Function

LPS Challenge. The results of the LPS challenge are reported as the change in rectal temperature (RT) post inoculation. The TM/VitE treatment had no effect ($P = 0.75$) on change in RT during the LPS challenge (Figure 4). Peak RT for both treatments occurred 3 h after LPS inoculation with a mean RT increase of 1.2 °C. A secondary increase (0.4 °C) in RT occurred 12 h after LPS inoculation. There was a transit-stress treatment x hour interaction ($P < 0.001$) for change in RT during LPS challenge (Figure 5). Rectal temperatures for both treatment groups peaked after 3 h with a mean RT increase of 1.2 °C. A secondary increase in RT was also observed 12 h after LPS inoculation, however, transit-stressed steers had a 300% greater ($P < 0.05$) increase in RT than non-transit-stressed steers at this time (0.8 °C vs 0.2 °C, respectively).

Humoral Immunity. Neither TM/VitE treatment nor transit-stress treatment affected humoral immunity. The effects of TM/VitE on humoral immunity are reported in Figure 6, while the effects of transit-stress treatment on humoral immunity are reported in Figure 7. There was evidence of a secondary immune response after the second injection given on d 35 when positivity values rose from 50% on d 35 to 60% by d 49.

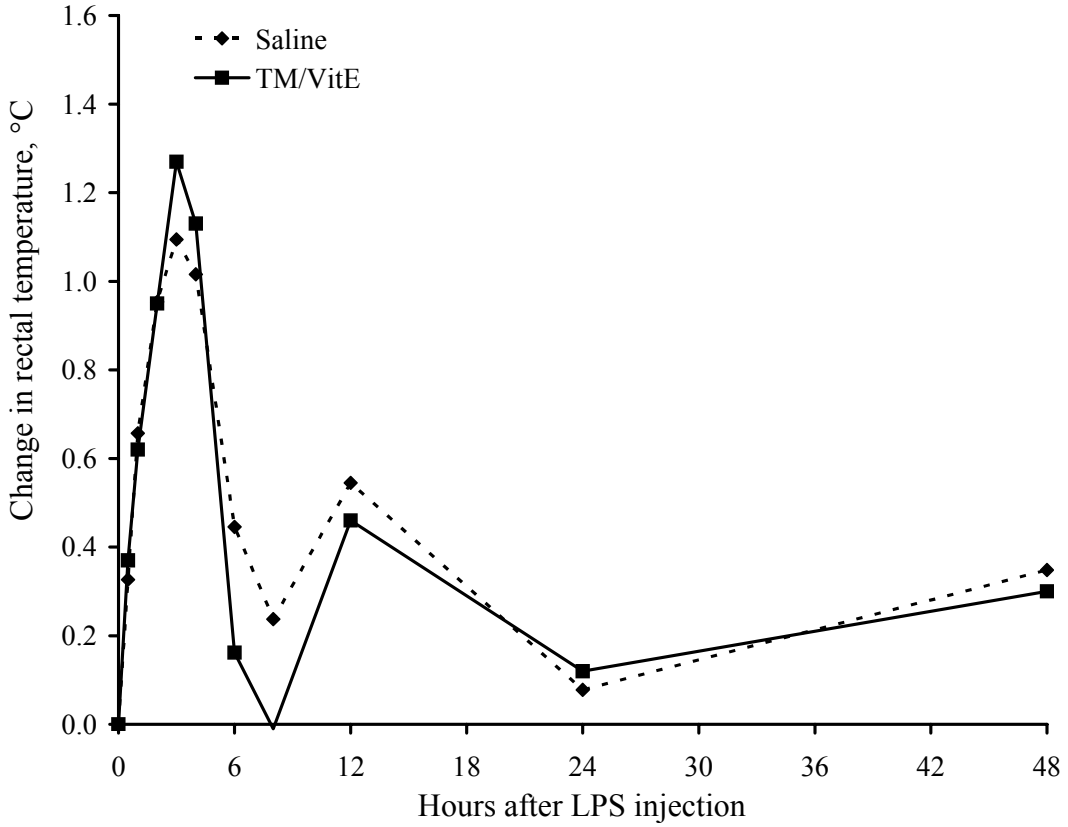

Figure 4. The effects of TM/VitE injections on change in rectal temperatures (RT) after lipopolysaccharide (LPS) injection in growing steers. There were no significant differences between treatment groups. TM/VitE effect ($P = 0.75$); hour ($P < 0.001$); TM/VitE x hour interaction ($P = 0.47$). There were no significant TM/VitE x transit-stress interactions.

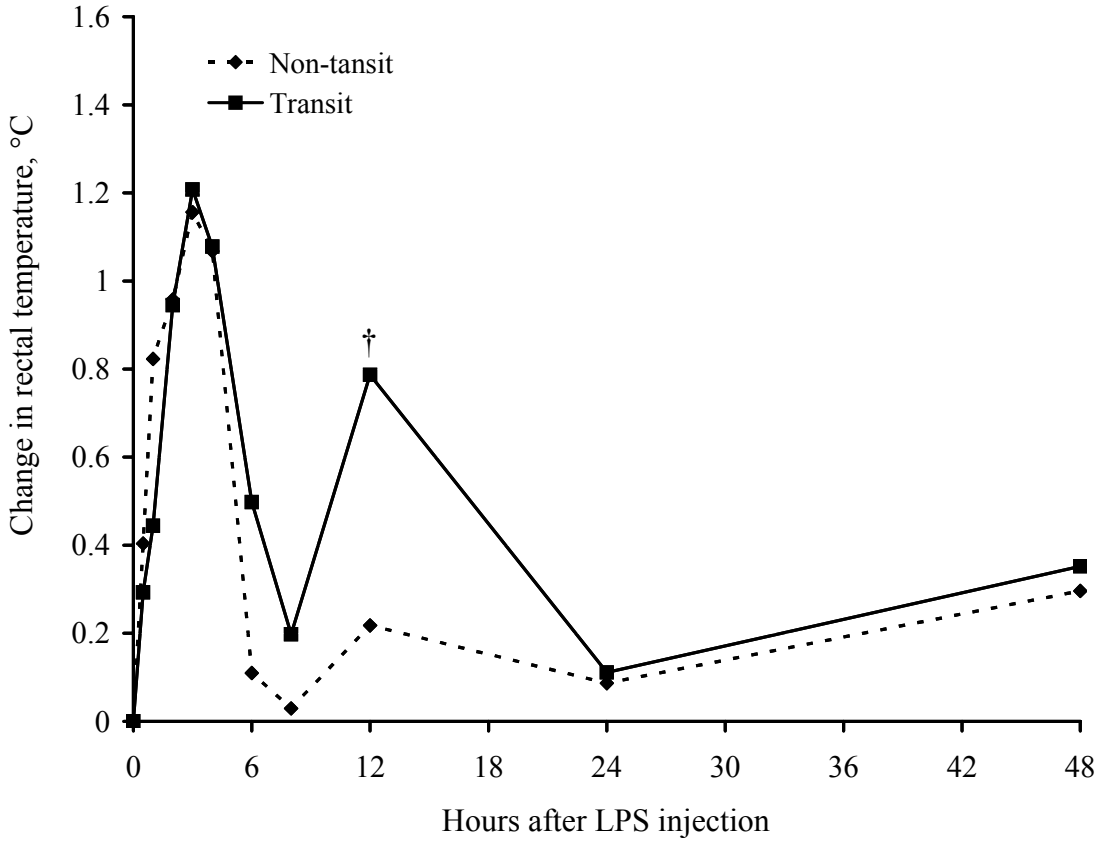

Figure 5. The effects of transit-stress treatment on rectal temperatures (RT) after lipopolysaccharide (LPS) injection in growing steers. Transit stress effect ($P = .044$); hour ($P < 0.001$); transit stress x hour interaction ($P < 0.001$). †Values are different ($P < 0.05$).

Cellular Immunity. Data taken from all steers on d 56 of the experiment showed abnormally hypersensitive skin-swelling response to the injected mitogens. Data from this day for all steers was removed from further analysis.

The effects of TM/VitE on PHA cellular immune responses on d 21 and 35 are reported in Figure 8. The TM/VitE-treated calves had 24% lower ($P < 0.02$) cellular immune responses to PHA compared to saline-injected steers on d 21. Treatment with TM/VitE did not have any affect on cellular immune response to PHA on d 35, but a TM/VitE treatment x transit-stress treatment x time ($P < 0.05$) interaction was observed. Mean separation of the TM/VitE x transit-stress sub-classes showed that no cellular immune responses to PHA were different at the $P = 0.05$ level on d 35.

The effects of transit stress treatment on PHA cellular immune for d 21 and 35 are reported in Figure 9. As expected, transit-stress treatment had no effect on cellular immune on d 21. Change in mean skin-fold thickness on d 21 peaked at 5 mm for transit- and non-transit-stressed steers after 4 h and steadily declined for the remainder of the 24-h period. Transit-stress treatment had no effect on PHA cellular immune response on d 35. The effects of TM/VitE treatment on cellular immune response to OVA are reported in Figure 10. TM/VitE treatment had no effect on cellular immune response to OVA on d 21, however a TM/VitE treatment x transit stress treatment x time ($P < 0.05$) interaction was observed. Mean separation of the TM/VitE treatment x transit-stress treatment classes showed that none were different at the $P = 0.05$ level. TM/VitE injection tended ($P = 0.18$) to decrease cellular immune response to OVA on d 21 with steers in the TM/VitE group averaging 30% lower responses as

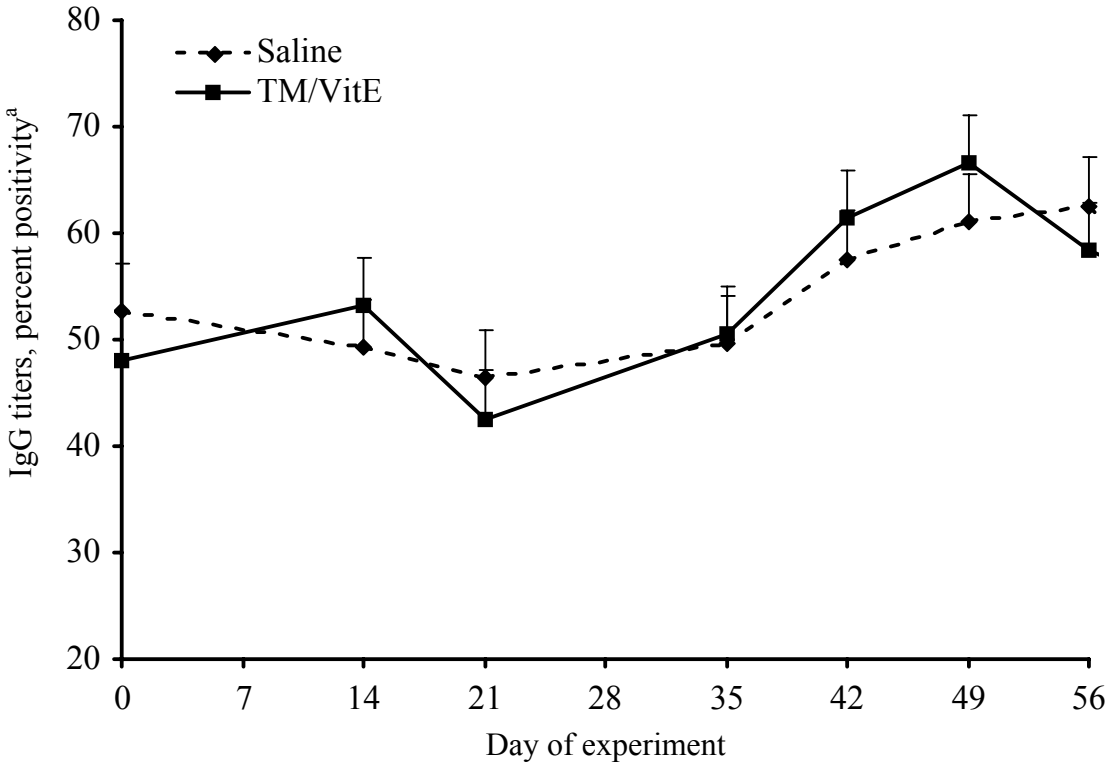

Figure 6. The effects of TM/VitE injections on antibody titer to keyhole limpet hemocyanin (KLH) in growing steers. [a]Value is expressed as a percentage of a positive control. Primary immunization given at d 0, with booster given on d 35. There were no significant differences between treatment groups on any days. TM/VitE effect ($P = 0.94$); day ($P < 0.0001$); TM/VitE x day interaction ($P = 0.07$). There were no significant TM/VitE x transit-stress interactions.

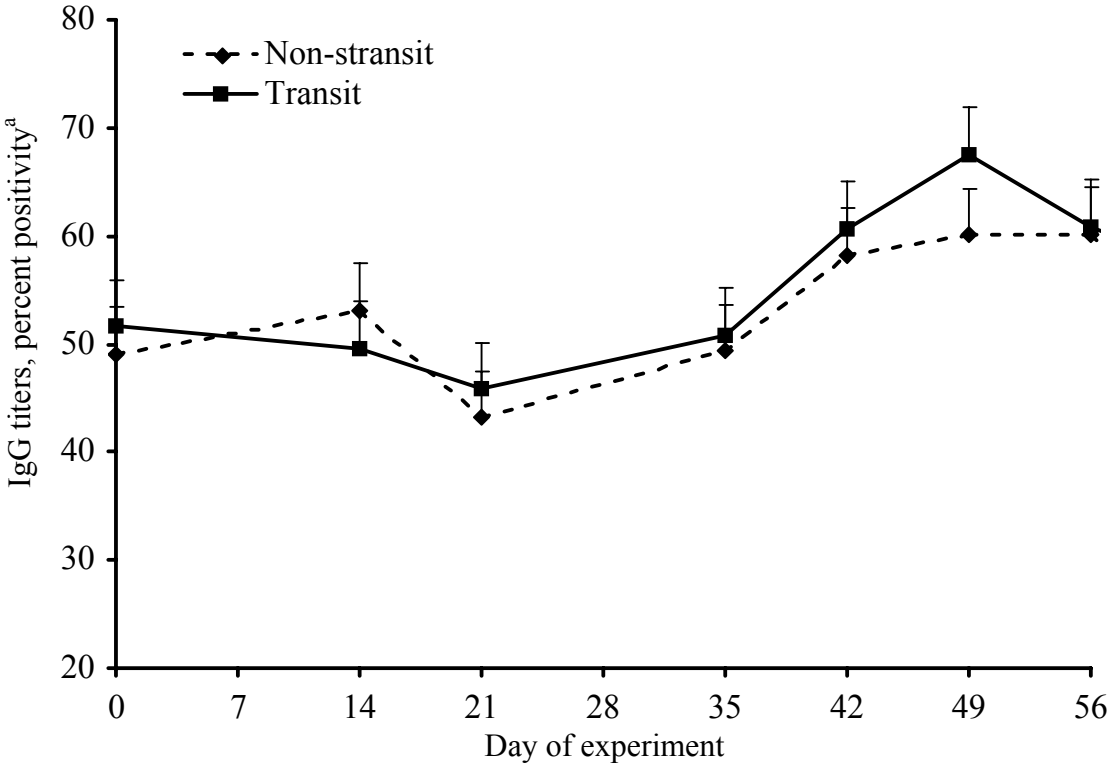

Figure 7. The effects of transit-stress treatment on antibody titer to keyhole limpet hemocyanin (KLH) in growing steers. [a]Value is expressed as a percentage of a positive control. Primary immunization given at d 0, with booster given on d 35. There were no significant differences between treatment groups on any days. Transit stress effect ($P = 0.35$); day ($P < 0.0001$); transit stress x day interaction ($P = 0.07$). There were no significant TM/VitE x transit stress interactions.

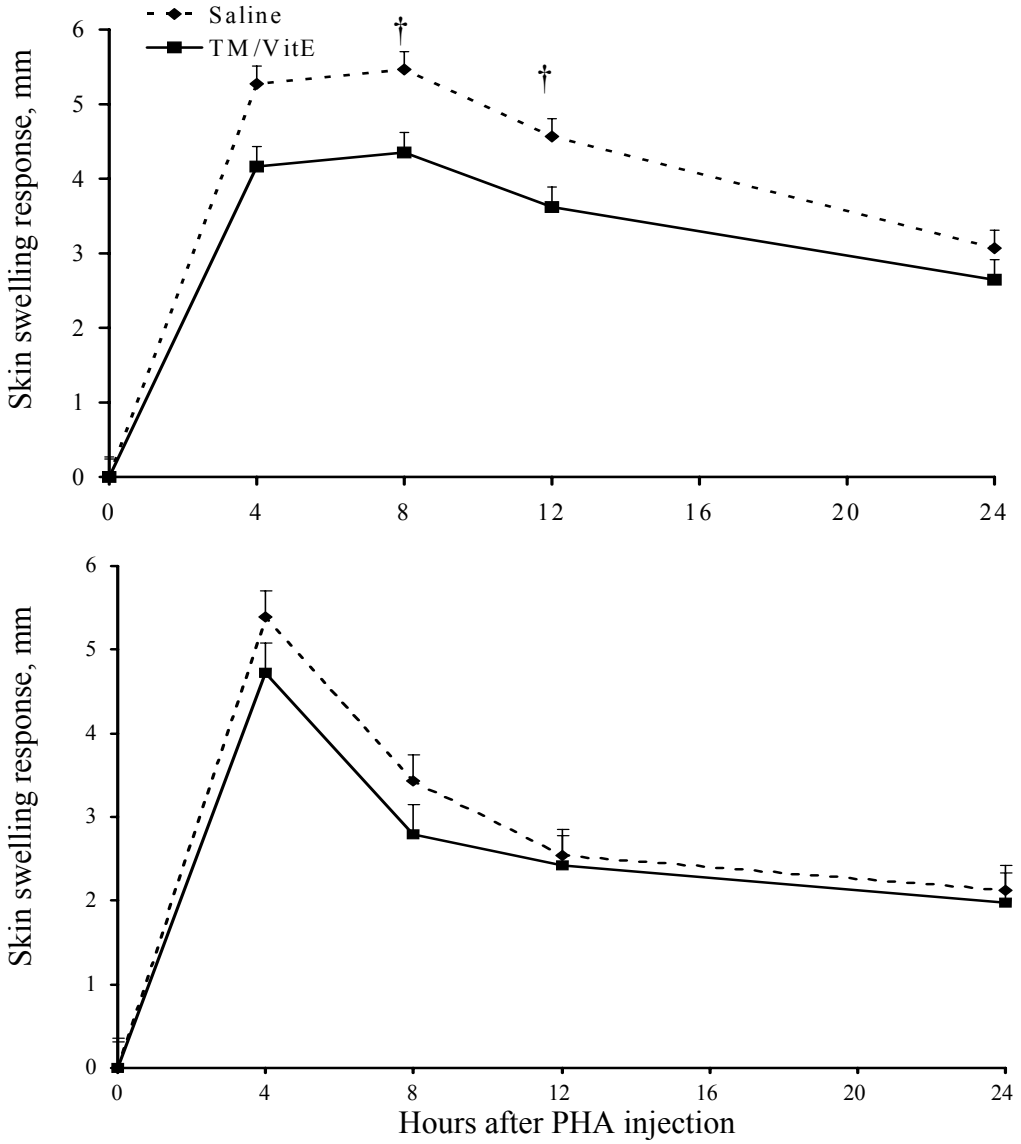

Figure 8. Effects of TM/VitE injections on phytohemmagglutinin (PHA)-induced skin-swelling responses on d 21 (top graph) and 35 (bottom graph) in growing steers. TM/VitE effect on d 21 ($P = 0.02$); Time ($P < 0.0001$); TM/VitE x time effect ($P = 0.43$). TM/VitE effect on d 35 ($P = 0.41$); Time ($P < 0.0001$); TM/VitE x time interaction ($P = 0.31$); there were no significant TM/VitE x transit stress interactions on d 21 or d 35. †Values are different ($P < 0.05$).

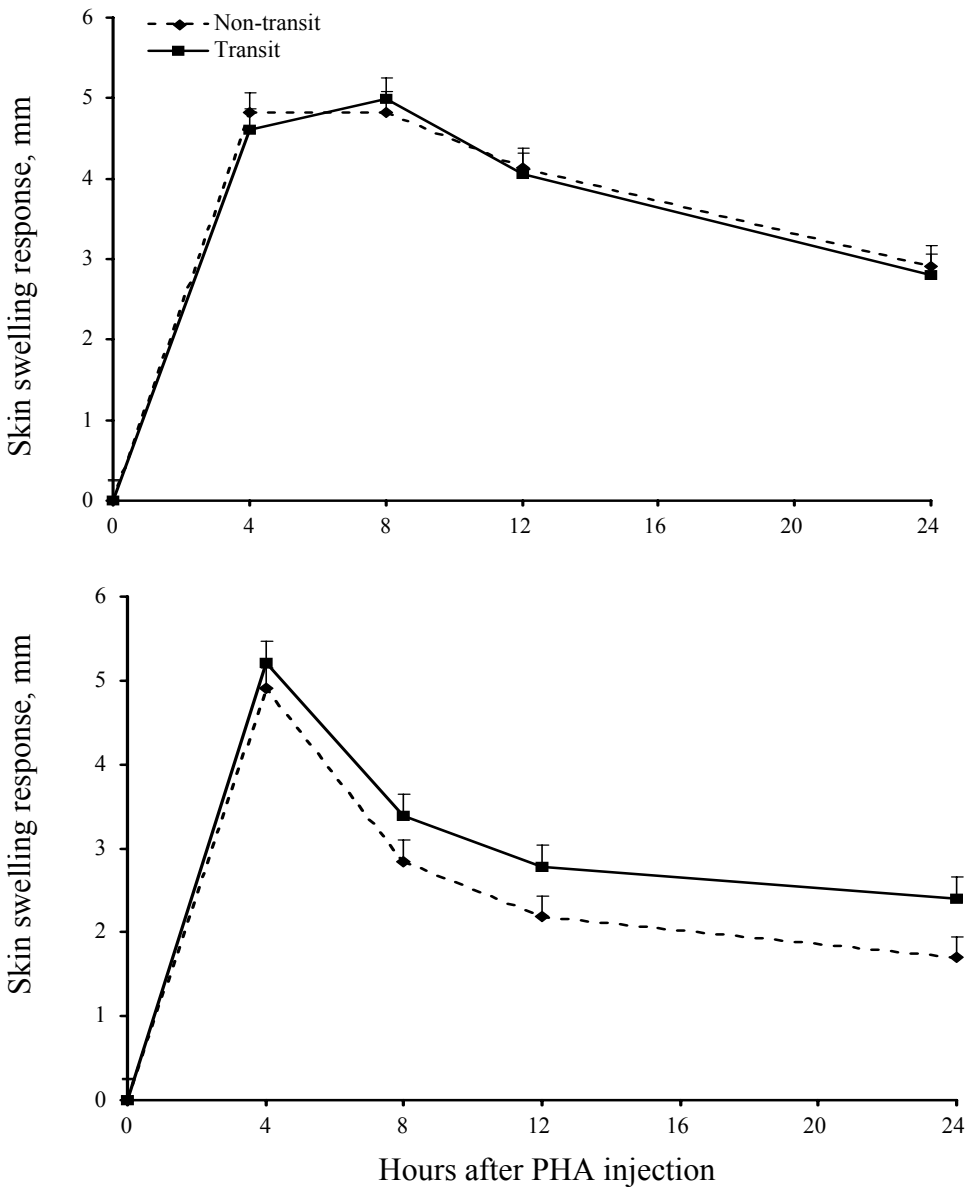

Figure 9. Effects of transit-stress treatment on phytohemmagglutinin (PHA)-induced skin-swelling responses on days 21 (top graph) and 35 (bottom graph) in growing steers. Transit stress effect on d 21 ($P = 0.87$); Time ($P < 0.001$); Transit stress x time interaction ($P = 0.71$). Transit stress effect on d 35 ($P = 0.21$); Time ($P < 0.001$); Transit stress x time effect ($P = 0.07$); there were no significant TM/VitE x transit stress interactions on d 21 or d 35, however there was a TM/VitE x transit stress x time interaction ($P < 0.05$) on d 35.

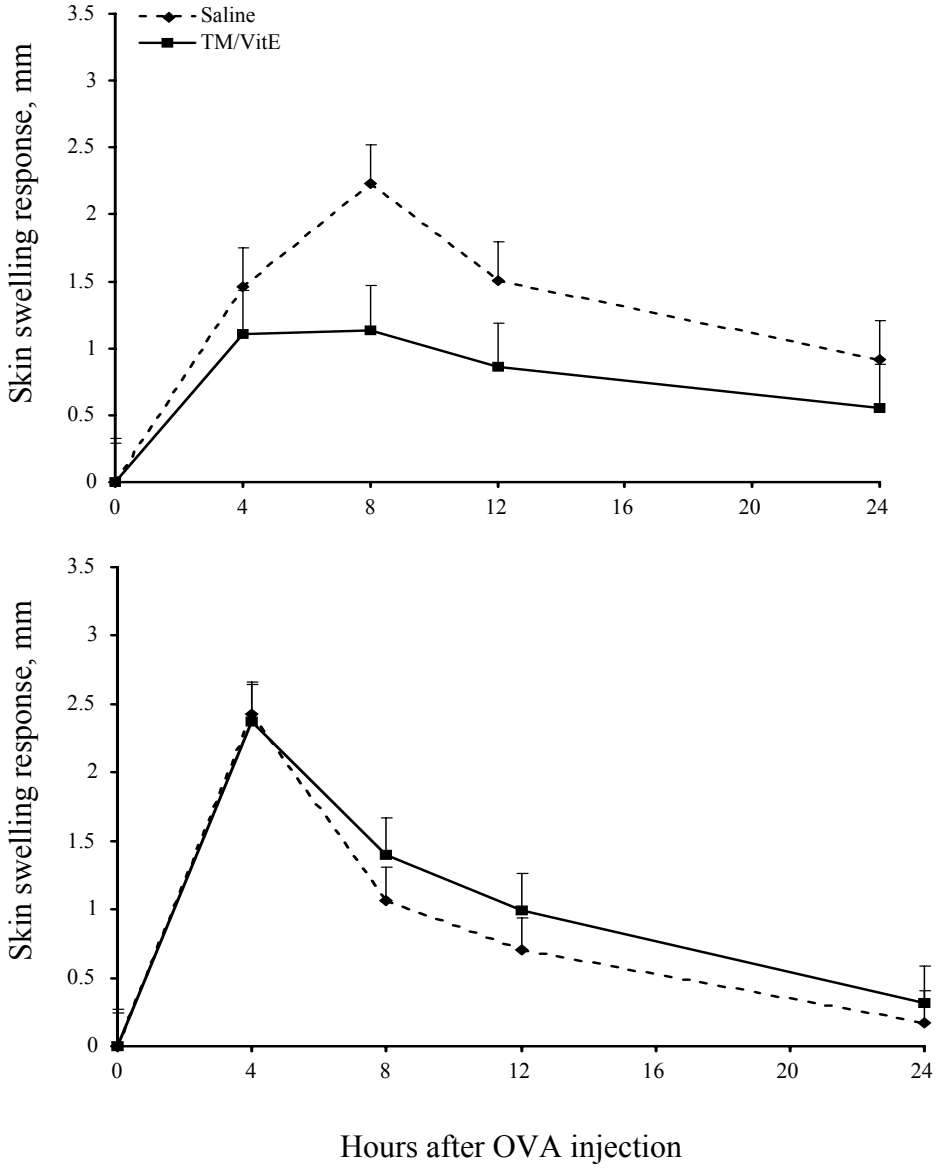

Figure 10. Effects of TM/VitE injections on ovalbumin (OVA)-induced skin-swelling responses on d 21 (top graph) and 35 (bottom graph) in growing steers. TM/VitE effect on d 21 ($P = 0.18$); Time ($P < 0.0001$); TM/VitE x time interaction ($P = 0.16$). TM/VitE effect on d 35 ($P = 0.62$); Time ($P < 0.0001$); TM/VitE x time interaction ($P = 0.47$); there were no significant TM/VitE x transit stress interactions on d 21 or d 35.

compared to saline-injected steers. The TM/VitE injection did not affect cellular immune response to OVA on d 35. Change in mean skin-fold thickness peaked at 2.4 mm for TM/VitE- and saline-injected steers after 4 hours and steadily declined for the remainder of the 24-h period.

Figure 11 shows the effect of transit-stress treatment on cellular immune responses to OVA on d 21 and 35. Transit-stress treatment did not affect cellular immune responses to OVA on d 21 or d 35. On d 21 the change in mean skin-fold thickness for all steers peaked at 2.3 mm after 4 hours and steadily declined over the 24-hour period. On d 35, the change in skin-fold thickness for all steers peaked at 2.3 mm after 4 h and steadily declined for the remainder of the 24-h period.

Lymphocyte Proliferation. Over the entire study, saline-injected steers had 40% higher ($P < 0.05$) lymphocyte proliferation to KLH compared to TM/VitE-injected steers (Figure 12). Transit-stress treatment had no effect ($P = 0.8$) on lymphocyte proliferation to KLH (Data not reported). Figure 13 shows the main effects of TM/VitE treatment on lymphocyte proliferation to OVA. Over the entire study, saline-injected steers had 70% higher ($P < 0.01$) lymphocyte proliferation response to OVA compared to TM/VitE-injected steers. Transit-stress treatment had no effect ($P = 0.25$) on lymphocyte proliferation to KLH (Data not reported). Neither TM/VitE injection ($P = 0.20$) nor transit-stress treatment ($P = 0.88$) had any effect on lymphocyte proliferation to PHA (Data not reported).

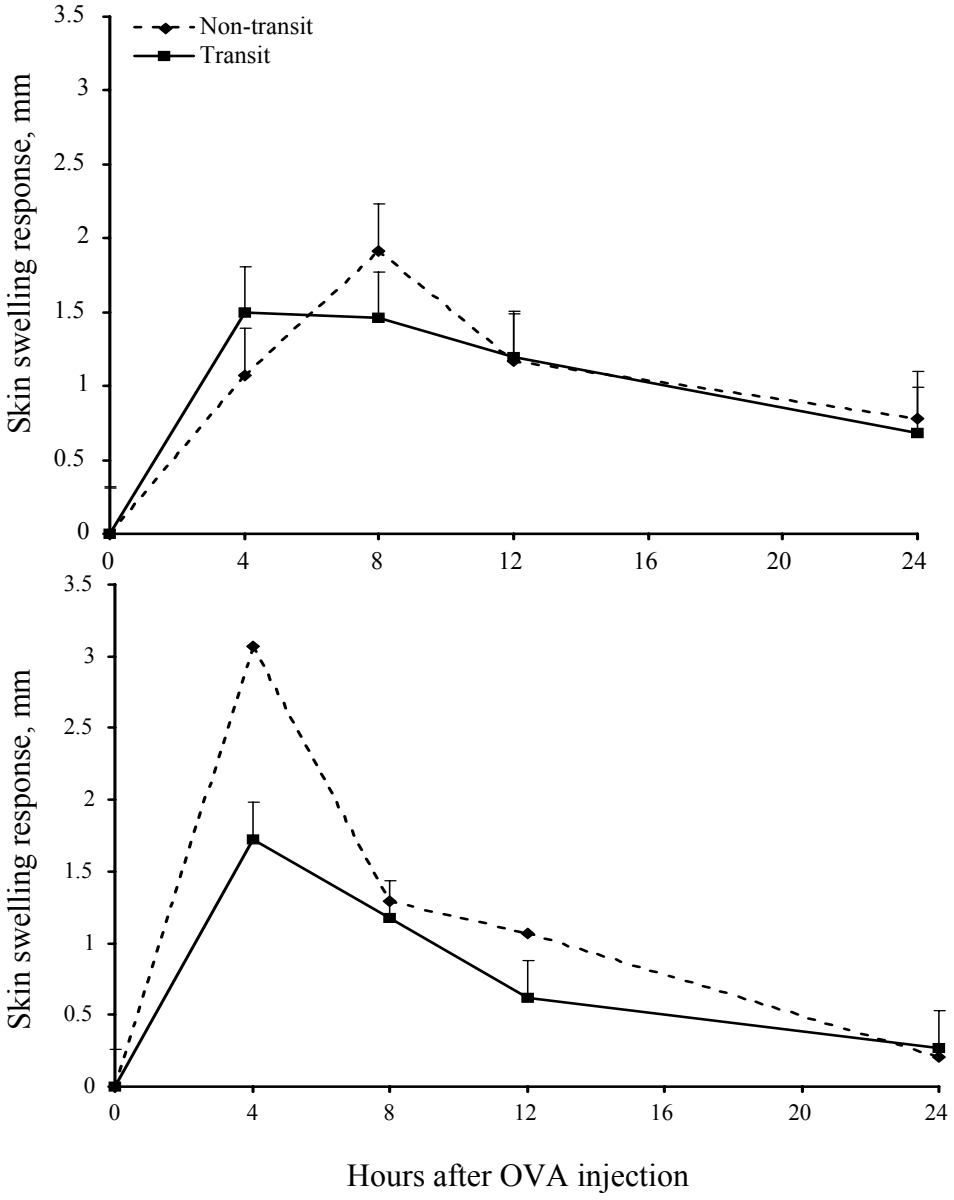

Figure 11. Effects of transit-stress treatment on ovalbumin (OVA)-induced skin-swelling responses on d 21 (top graph) and 35 (bottom graph) in growing steers. Transit stress effect on d 21 ($P = 0.96$); Time ($P < 0.0001$); Transit stress x time effect ($P = 0.03$). Transit stress effect on d 35 ($P = 0.21$); Time ($P < 0.0001$); Transit stress x time interaction ($P = 0.07$); there were no significant TM/VitE x transit stress interactions on d 21 or d 35, however, there was a TM/VitE x transit stress x time interaction ($P < 0.05$) on d 21.

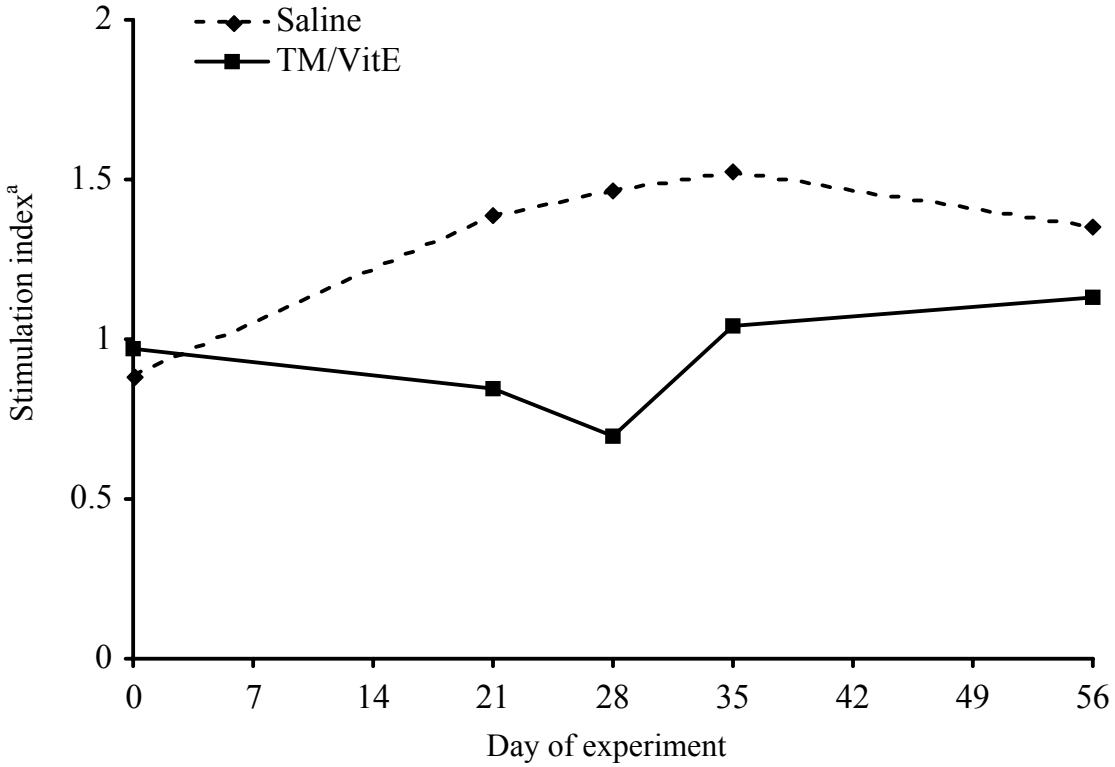

Figure 12. Effects of TM/VitE injections on lymphocyte proliferation to keyhole limpet hemocyanin (KLH) in growing steers. [a]Data reported as stimulation index (S.I.) calculated by dividing the sample cell counts by negative control cell counts. TM/VitE effect ($P = 0.03$); day ($P < 0.0001$); TM/VitE x day interaction ($P = 0.29$). There were no significant TM/VitE x transit-stress interactions.

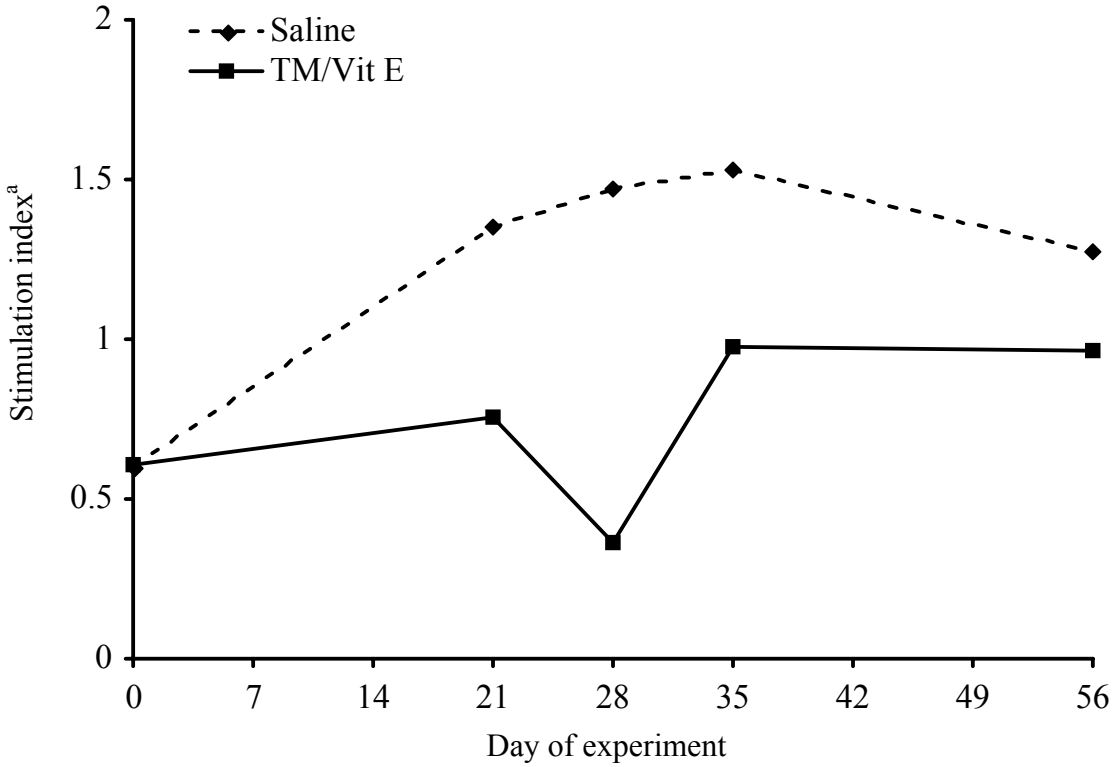

Figure 13. Effects of transit-stress treatment lymphocyte proliferation to ovalbumin (OVA) in growing steers. [a]Data reported as stimulation index (S.I.) calculated by dividing the sample cell counts by negative control cell counts. Transit stress effect ($P < 0.01$); day ($P < 0.0001$); transit stress x day interaction ($P = 0.09$). There were no significant TM/VitE x transit stress interactions.

Discussion

Calf Performance

Treatment with Multimin^{TM} Cu, Zn, Se, and Mn supplement, and vitamin E, reduced ADG and feed efficiency during the first 24 d of the experiment. TM/VitE injections minimally effected the percent BW change during a transit stress experiment and LPS challenge. Chirase et al. (1994) found that steers fed dietary Zn (to provide 225 mg Zn/d) for 18 d and injected with 120 mg of Cu glycinate 11 d prior to transit (2500 km) consumed 31% less feed than steers feed supplemental Zn, but not injected with Cu glycenate. Moreover, the Cu-injected steers lost more BW and regained BW slower than non-injected steers. Wright et al. (1990) reported that supplementation with dietary Cu, Se, and Vitamin E did not affect performance of steers. Davis (1999) reported no change in BW, DMI, or ADG at any time for calves fed various dietary Cu concentrations up to 80 ppm during the post-weaning period. Wikse et al. (1992) suggested that growth might not be inhibited due to Cu deficiency unless animals are severely Cu deficient (< 15 ppm DM liver Cu) for an extended period of time. The steers in the current study did not approach what would be considered deficiency status for either of the trace minerals examined.

Transit-stress treatment lowered BW and transit-stressed steers lost a greater percentage of total BW during transit than non-transit-stressed steers. It must be noted that the transported steers received limited intake and non-transit-stressed steers endured harsh weather conditions during the transit-stress period. The difference in change of BW percentage from d 24 to d 31 was most likely influenced by the fact that the

transported steers were fasted during the transit-stress period. Transit-stress treatment had no effect on ADG, DMI or feed efficiency either prior to or after transportation. This did not agree with Kegley et al. (1997) who reported that for the first three d post-IBRV challenge, transported steers showed decreased DMI compared to non-transported steers. Subsequently, on d 17 through d 19 post-IBRV inoculation, transported steers showed increased DMI compared to non-transported steers.

Liver Mineral Status

Trace mineral injections on d 0 and 21, increased Cu concentrations in livers of the TM/VitE-injected animals. At the beginning of the experiment, liver Cu for all calves averaged 82.9 mg/kg DM, which fell within the adequate range of 25 to 100 mg/kg DM (Underwood and Suttle, 1999). By the end of the experiment, mean liver Cu concentrations had increased to 84.89 mg/kg DM in the TM/VitE-injected steers, whereas, liver Cu concentrations of saline-injected steers had decreased to 29.47 mg/kg DM. The decrease in liver Cu was expected as additional S and Fe were added to partially impair Cu absorption. Bremner et al. (1987) reported that steer calves feed a diet high in Fe showed reduced liver Cu concentrations, and that introducing S to the diet further decreased liver Cu concentrations.

Liver Zn levels were adequate to high throughout the experiment for both TM/VitE-injected and saline-injected steers. Liver Zn concentration for both treatment groups was similar on d 0 and averaged 128 mg/kg DM. TM/VitE-supplemented steers had 135 and 188% higher liver Zn concentration on d 30 and 56, repectively. Even though supplemented steers had higher liver Zn concentrations on d 30, both groups

finished the experiment with liver Zn concentrations above those considered normal (normal range 25 to 100 mg/kg DM). Studies by Puls (1994) and Engle et al. (1997) suggest that tissue Zn level may be a poor indicator of overall Zn status. These studies showed no differences in liver or plasma Zn between experimental groups fed Zn depletion and Zn repletion diets, even when performance was inhibited. Even though TM/VitE-injected calves had higher liver Zn on d 30; saline-injected calves still maintained adequate liver Zn concentrations.

Transit-stress treatment had little effect on liver mineral status. Transit stress has been shown to acutely increase serum cortisol levels with peak cortisol concentrations occurring about 2 h following stress initiation (Kent and Ewebank, 1983). Studies have shown that increases in plasma cortisol cause a concurrent increase in urinary (Henkin, 1974) and biliary (Mearrick and Mistilis, 1969) Cu excretion. One explanation for the lack of this evidence in the present experiment could be that non-transported cattle endured stress related to harsh weather conditions during the transit stress phase (rain and a 20 °C temperature drop) while in College Station. The transported calves did not endure such a temperature difference, and spent 70% of the trip in dry, calm weather conditions. The only difference between treatment groups occurred on d 56 when transported steers, had higher liver Cu concentrations (64.6 vs 49.7 mg/kg DM) than non-transported steers. For this reason, it is possible that the non-transported calves were stressed to the same extent as the transported calves.

No differences in liver Zn concentrations were observed between transit-stressed or non-transit-stressed calves on any of the observation days. Using Zn

depletion/repletion trials, Suttle et al. (1982) reported that Zn levels were controlled by absorption and uptake, and not by excretory pathways. The presence of adequate initial Zn levels, and the fact that Zn supplementation occurred via s.c. injection, could explain the lack of differences seen between transit-stress treatment groups.

Serum Mineral Status

Serum mineral concentrations are commonly lower than plasma concentrations due to the possible absence of many mineral-containing proteins. Serum Cu was well above the adequate levels for plasma Cu observed in Puls (1994) for the length of the experiment. Despite the saline-injected steers having low liver Cu levels of (29.7 mg/kg DM) by d 56, serum Cu levels were not depressed (mean d 56 serum Cu for all calves, 0.62 mg/L) as severely as Claypool et al. (1975) reported when liver Cu levels below 40 mg/kg consistently lowered plasma Cu levels below 0.6 mg/L. TM/VitE-injected steers had higher serum Cu concentrations than saline-injected steers on d 24 and 26. During this time period, the steers endured stressful conditions (half were transit-stressed, and the other half endured severe weather). This agrees with research that serum Cu concentrations increase during periods of stress or infection (Orr et al., 1990; Chirase et al., 1991).

Serum Zn status was not different for either TM/VitE-injected or saline-injected calves for all days except d 24, when saline-injected steers had higher serum Zn concentrations than TM/VitE (1.1 mg/L vs 0.8 mg/L, respectively). The fact that liver Zn concentrations were well above adequate, and that increased variation has been found

between individual animal plasma Zn concentrations (Blackmon et al., 1967), could negate the significance of this observation.

Multimin^{TM} injection provided s.c. selenium in the form of sodium selenite. Enjalbert et al. (1999) fed 72 Salers cows 2.5 and 45.5 mg/d selenium as sodium selenite. The authors reported that sodium selenite was a highly available source and that increasing dietary sodium selinite increased plasma glutathione peroxidase activity. In the current study, whole blood Se concentration was well above adequate for all treatment groups, and a difference between TM/VitE- and saline-injected steers didn't occur until d 56 of the experiment, when saline-injected steers (228.9 μg/mL) had higher Se concentration than TM/VitE-injected steers (182.3 μg/mL), still well above adequate.

Random assignment of transit-stress treatment resulted in transit-stressed steers having higher serum Cu concentrations than non-transit-stressed steers on d 0 (prior to injection with Multimin). These values had equalized by d 24 (prior to transit departure). Upon return from transit (d 26), transported steers had higher serum Cu concentration than non-transported steers (1.1 vs. 0.9 mg/L). It should be noted that the steers that were not transit-stressed endured harsh cold temperatures and thunderstorms while remaining in their pens in College Station. Without analyzing the change in cortisol levels across the treatments, it was difficult to determine whether this difference was caused by an induced stress response, or which group (transported vs. non-transported) had been stressed to a higher extent. The increase in serum Cu could be caused by the increased mobilization from tissue stores due to increased Cu excretion during catabolic and stress episodes (Cousins, 1985; Orr et al., 1990).

Serum Zn was not different across transit-stress treatments and was well above adequate for the length of the experiment. Random assignment of transit-stress treatment resulted in transit-stressed steers having higher whole blood Se concentration (115.1 μg/mL) than non-transit stressed calves (93.5 μg/mL). These values equalized by d 24 and no difference was seen for the remainder of the experiment. Conversely, Nockels et al. (1993) reported a decrease in serum Zn concentration in charolais steers that underwent an induced (adrenocorticotropic hormone injection) market stress challenge for 3 d.

Serum Vitamins

All calves had serum α-tocopherol levels of 2.5 μg/mL on d 0, and by d 24 TM/VitE injection had increased α-tocopherol levels to 7.69 μg/mL, while concentrations from saline-injected steers remained unchanged. As expected, TM/VitE-injected animals maintained higher serum α-tocopherol levels throughout the remainder of the experiment.

Saline-injected steers possessed increased serum γ-tocopherol on d 24 and d 26 compared to TM/VitE-injected steers. The form of vitamin E supplemented in the injections was α-tocopherol, so the γ-tocopherol levels were not expected to follow treatment lines. Vitamin A was not supplemented, but was analyzed in the experiment. The serum vitamin A concentrations were the same for both treatment groups for all days except d 26, when saline-injected steers had higher serum vitamin A concentrations than TM/VitE-injected steers (0.2 μg/mL vs. 0.13μg/mL, respectively).

All calves, regardless of transit stress treatment, had the same α-tocopherol concentration at departure (mean serum α-tocopherol 5.3 μg/mL) and had decreased concentrations upon return (mean serum α-tocopherol 4.2 μg/mL) from transit stress. This observation could be explained by the adverse weather conditions that the non-shipped animals experienced. Serum α-tocopherol concentrations were increased for non-transit-stressed steers compared to transit-stressed steers only on d 56 of the experiment. It has been shown that transit stress can significantly reduce the serum concentrations of vitamin E (Chirase et al., 2001). In that study, animals were shipped from Tennessee to Texas (approximately 2500 km) and divided into the following stress groups: 1) simulated dust environment tent, and 2) tent without dust simulation. Transit stress decreased vitamin E (α-tocopherol) concentration from 7.1 to 1.95 μg/mL (below the critical 2 μg/mL). This agreed with Nockels et al. (1996) who observed a decrease in serum vitamin E levels when non-supplemented steers were subject to stress as compared to vitamin E supplemented steers. Serum α-tocopherol concentration in the current study never fell below the crucial 2 μg/mL concentration. Transit stress also lowered serum γ-tocopherol on d 26 and 35 and lowered vitamin A concentration on d 26.

LPS Challenge

Steers from both TM/VitE and saline-injected treatments showed increased RT in response to LPS inoculation. Rectal temperature plots for both treatments groups were bimodal with a large peak after 3 h (1.2 °C) and a second, less intense, peak after 12 h

(0.5 °C). Transit-stress had little effect on change in RT. Transit-stressed steers had an increased change in RT compared to non-transit-stressed steers 12 h after LPS inoculation. The increases in RT of LPS challenged steers is similar to the results of Elsasser et al. (1996) who reported that mean change in basal RT of LPS inoculated calves peaked between 3 and 4 h after inoculation at a value between 1.2 and 1.4 °C. The authors stated that LPS was a valuable tool used to examine disease processes because it produced a gradable response that was proportional to injected dosage. Fever was an important characteristic of the endotoxin administration, and the febrile response has been used as an accurate method to quantify the severity of the elicited condition.

Humoral Immunity

Treatment with TM/VitE had no effect on antibody titer response to KLH. Though it is well documented in rodent studies that Cu-deficiencies cause increased numbers of splenic B cells due to thymic atrophy (Lukasewycz et al., 1985; Bala et al., 1990; Prohaska and Lukasewycz, 1983), humoral immune function studies in cattle have been more variable. Past studies have shown that antibody titers to an antigen are impaired (Cerone et al., 1995), unchanged (Ward et al, 1993; Niederman et al., 1994) or elevated (Stabel et al., 1993) in Cu-deficient steers when compared to Cu-adequate steers. Cerone et al. (1995) reported decreased antibody titers to *B. abortus* in steers with plasma Cu concentrations below 0.5 mg/L as compared to steers with adequate plasma Cu (> 0.9 mg/L). Stable et al. (1993) reported a decrease in serum IgM in Cu-deficient steers (< 0.4 mg/L plasma Cu) compared to Cu-adequate (> 0.8 mg/L plasma

Cu), however antibody titers to IBRV were elevated in the Cu deficient steers compared to Cu adequate steers.

Transit-stress treatment had no effect on humoral immune function in growing steers. Brown-Borg et al. (1993) reported that young pigs with high baseline levels of plasma cortisol had a lowered immune response compared to pigs with low baseline cortisol levels. The authors suggested the possibility of high/low stress predestination, which would influence immune function and favor those "low stress" animals over "high stress" animals. Without determination of base cortisol levels, or peak levels during transit stress, there was no way to determine exactly how much or a stress response was induced.

Cellular Immunity

Treatment with TM/VitE injections did not enhance cellular immune responses in growing steers during this study. On d 21, saline-injected cattle had higher PHA-induced skin swelling responses (5.3 vs 4.1 mm) compared to TM/VitE steers. The PHA-induced responses on d 35 and OVA-induced skin swelling responses on d 21 and 35 were similar for TM/VitE- and saline-injected steers.

In rodents, it has been shown that Cu-deficiency reduced the number of Th cells and Tc cells in both mice and rats (Bala et al., 1991; Mulhern and Koller, 1988; and Lukasewycz et al., 1985). Bala and Failla (1992) also reported a decrease in the proliferative responses of T cells to mitogen during Cu-deficiency, and suggested that this was caused by the inability of Th-1 cells to produce adequate IL-2.

Evidence of a clear mechanism for trace mineral effects on cellular immune function has been less evident in cattle. Ward and Spears (1999) reported that dietary Cu treatment had minimal effect on delayed-type hypersensitivity (DTH). Branum (1999) reported that calves born to cows which were supplemented prenatally with 80 ppm dietary Cu, had increased skin-swelling responses compared to calves born to cows supplemented with either 0, 20, or 40 ppm dietary Cu. The author suggested that the immune function of calves may have been dependent upon Cu status of the dam, and differences observed could have been due to proper development of primary and secondary immune organs. In the current experiment, perhaps, either 1) trace mineral deficiency was not severe enough to elicit any type of immune dysfunction, or 2) the transient, marginal deficiency induced in saline-injected steers at the end of the study was not severe enough to elicit immune dysfunction due to previous proper development of the immune organs.

Transit stress treatment did not affect PHA or OVA cellular immune responses on any of the observation days. Ward and Spears (1999) transported angus bulls on an industrial trailer for 2.5 h. The bulls remained on the trailer for 9.5 h in order to bring the total time on the trailer to 12 h. Bulls that were fed supplemental Cu and transit-stressed showed increased DTH response compared to non-stressed animals. These results disagree with reports that transit stress impairs immune function (Kelly, 1988). Kegley et al. (1997) reported no differences in percentages of neutrophils or in ratio of neutrophils to lymphocytes between transit-stressed and non-transit-stressed steers 7 d after transit stress. The steers in the current study were evaluated for cellular immune

responses on d 21 and 35, and were transit-stressed between d 24 and 26 (9 d after return from stress).

Lymphocyte Proliferation

Treatment with TM/VitE decreased lymphocyte proliferation to KLH and to OVA, but had no effect on proliferative response to PHA. The lymphocyte proliferation assay is an *in vitro* evaluation of the cellular immune response, and the delayed-type hypersensitivity response measurement (skin-swelling response) is an *in vivo* evaluation of cellular immunity. TM/VitE injection suppressed PHA cellular immune response on d 21, and lymphocyte proliferation to PHA on d 21 is numerically higher, though the difference failed to reach significance. Arthington et al. (1995) reported no differences in lymphocyte blasotgenesis when Cu-deficient heifers were repleted with Cu. Stabel et al. (1990) added sodium selenite to bovine lymphocytes after they were incubated with Con-A, PHA, or PWM. Optimal enhancement of stimulation to PWM occurred with the addition of 50 to 400 ng $Na_2SeO_3 \bullet mL^{-1}$ to the culture medium while the responses to the T cell mitogens, Con A and PHA were minimally affected by Se repletion. Researchers suggested that the enhancement of lymphocyte proliferation in PWM cultures resulted from increased production of cytokines such as IL-1 and IL-2 by Th cells.

CHAPTER IV

CONCLUSIONS

Treatment with Multimin^{TM} and vitamin E increased liver Cu and Zn concentration and increased serum α-tocopherol concentration in growing steers fed a diet containing S (0.57%) and Fe (1500 ppm). Trace mineral supplementation decreased growth and feed efficiency, suggesting that the dose administered in this study was too high. Many studies have examined the effects of trace mineral and vitamin E supplementation on immune function; however, few have used injectable multi-source supplements. While humoral immunity was not affected by TM/VitE treatment, cellular immune function was decreased. TM/VitE-injected steers had decreased lymphocyte proliferation to KLH and OVA over the entire study and also had decreased skin-swelling responses to PHA on d 21, but not d 35. Transit stress treatment did not affect liver or serum mineral status, but did acutely lower serum vitamin A and γ-tocopherol levels. Both humoral and cellular immune functions were unaffected by transit stress. Trace mineral supplementation did increase the trace mineral status of the steers in this study, however, the decreases in performance and immune function could be due to the fact that steers in the experiment were not initially deficient in Cu, Zn, Se or vitamin E, and that further supplementation elicited toxic effects.

The financial losses associated with the reduced ADG and feed efficiency reported in this experiment would be substantial when applied to feedlot economics. The use of an injectable multi-source trace mineral supplement would logistically increase the efficiency of feedlot management practices; however, the effects of using

these supplements are extremely inconsistent. Additional research is needed to better assess trace mineral status of animal in order to establish adequate doses of Cu, Zn, Se and Mn to elicit optimal physiological responses such as growth and immune function.

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APPENDIX

Table A-1. Individual BW (kg) of growing steers throughout experiment.

Id	Trt	Tran	D0	D7	D14	D21	D24	D26
121	Sal	No	244	244	257	269	288	265
126	Sal	No	220	213	220	230	224	239
128	Sal	No	251	258	259	278	280	287
132	Sal	No	262	266	269	286	296	298
135	Sal	No	229	232	251	253	259	269
139	Sal	No	216	231	234	251	268	264
140	Sal	No	247	249	265	282	292	288
122	Sal	Yes	221	236	230	256	262	227
127	Sal	Yes	250	269	267	279	306	259
129	Sal	Yes	266	269	280	281	279	265
136	Sal	Yes	231	252	262	267	286	270
142	Sal	Yes	192	200	215	214	227	201
143	Sal	Yes	239	231	241	249	255	235
130	TM/E	No	239	230	245	252	262	268
131	TM/E	No	227	212	216	238	240	241
134	TM/E	No	253	257	261	269	265	274
145	TM/E	No	216	213	222	225	232	241
147	TM/E	No	230	227	233	239	232	245
123	TM/E	Yes	230	230	242	259	247	240
124	TM/E	Yes	250	247	259	258	259	248
125	TM/E	Yes	216	220	227	230	235	215
133	TM/E	Yes	218	205	213	230	225	212
141	TM/E	Yes	253	259	254	269	275	257
146	TM/E	Yes	239	247	261	264	267	246

Table A-1 (Cont.).

Id	Trt	Tran	D30	D31	D35	D42	D49	D56
121	Sal	No	259	270	269	294	309	317
126	Sal	No	219	212	223	241	239	259
128	Sal	No	273	275	282	297	308	322
132	Sal	No	280	294	292	299	319	330
135	Sal	No	234	245	251	261	272	279
139	Sal	No	241	250	259	269	288	284
140	Sal	No	260	256	271	288	295	309
122	Sal	Yes	221	219	226	238	242	247
127	Sal	Yes	258	259	281	292	290	303
129	Sal	Yes	261	273	286	303	319	322
136	Sal	Yes	259	263	275	286	295	308
142	Sal	Yes	211	208	207	224	237	236
143	Sal	Yes	185	234	249	247	263	265
130	TM/E	No	254	261	259	276	283	288
131	TM/E	No	223	230	230	253	261	269
134	TM/E	No	259	269	274	283	295	299
145	TM/E	No	213	225	230	242	243	249
147	TM/E	No	220	229	242	258	266	267
123	TM/E	Yes	.	.	.	.	.	.
124	TM/E	Yes	220	215	244	256	260	269
125	TM/E	Yes	210	212	230	239	244	250
133	TM/E	Yes	199	201	214	209	227	228
141	TM/E	Yes	259	259	274	286	296	296
146	TM/E	Yes	259	257	259	269	274	278

Table A-2. Individual feed intake (kg, as fed basis) of steer throughout the experiment.

Id	Trt	Tran	D7	D14	D21	22-24	31-35	D42	D49	D56
121	Sal	No	57	61	71	31	51	84	89	86
126	Sal	No	50	46	52	25	35	62	64	71
128	Sal	No	54	52	64	26	45	78	80	83
132	Sal	No	50	53	69	30	51	79	80	79
135	Sal	No	60	68	68	31	40	61	62	70
139	Sal	No	52	50	63	27	48	74	66	69
140	Sal	No	54	65	80	34	43	75	84	87
122	Sal	Yes	53	54	70	30	27	41	48	54
127	Sal	Yes	54	50	61	27	38	57	56	63
129	Sal	Yes	53	55	65	28	44	72	79	74
136	Sal	Yes	58	70	76	31	39	77	83	88
142	Sal	Yes	61	73	80	33	51	85	89	78
143	Sal	Yes	51	47	45	20	31	51	53	52
130	TM/E	No	44	43	52	25	45	65	66	66
131	TM/E	No	50	45	57	26	36	61	64	73
134	TM/E	No	53	54	61	27	47	71	69	72
145	TM/E	No	51	46	51	24	45	62	59	59
147	TM/E	No	51	51	55	23	35	60	65	67
123	TM/E	Yes	50	50	58	23	.	.	.	.
124	TM/E	Yes	53	57	59	29	36	47	52	59
125	TM/E	Yes	54	58	55	23	35	57	55	59
133	TM/E	Yes	53	61	64	25	34	56	55	58
141	TM/E	Yes	55	51	65	32	39	76	80	80
146	TM/E	Yes	56	61	67	30	36	56	59	55

Table A-3. Raw data values for liver Cu concentrations (mg/kg DM) in growing steers.

Id	Trt	Tran	Day 0	Day 30	Day 56
121	Sal	No	77.75	60.53	21.27
126	Sal	No	52.39	27.28	11.75
128	Sal	No	94.38	52.28	18.92
132	Sal	No	61.00	43.38	13.32
135	Sal	No	95.27	54.59	50.95
139	Sal	No	98.79	71.90	31.63
140	Sal	No	80.98	61.07	26.85
122	Sal	Yes	81.12	48.11	30.71
127	Sal	Yes	85.89	64.99	39.59
129	Sal	Yes	73.39	62.11	17.17
136	Sal	Yes	66.41	53.66	20.45
142	Sal	Yes	70.15	65.50	31.92
143	Sal	Yes	93.05	87.40	60.94
130	TM/E	No	55.00	108.27	74.93
131	TM/E	No	143.70	152.37	76.58
134	TM/E	No	97.20	105.20	86.14
145	TM/E	No	72.13	172.27	64.06
147	TM/E	No	36.10	122.01	64.60
123	TM/E	Yes	87.31	127.40	96.50
124	TM/E	Yes	74.22	184.57	99.26
125	TM/E	Yes	156.94	119.49	90.75
133	TM/E	Yes	75.60	122.10	80.71
141	TM/E	Yes	70.71	158.69	116.46
146	TM/E	Yes	113.21	60.53	21.27

Table A-4. Raw data values for liver Zn (mg/kg DM) concentrations in growing steers.

Id	Trt	Tran	Day 0	Day 30	Day 56
121	Sal	No	131.54	111.50	98.51
126	Sal	No	125.43	147.02	98.75
128	Sal	No	125.66	117.48	105.14
132	Sal	No	122.31	109.80	123.44
135	Sal	No	128.98	123.65	120.34
139	Sal	No	124.55	106.54	95.40
140	Sal	No	123.29	146.09	79.14
122	Sal	Yes	122.56	142.38	97.07
127	Sal	Yes	164.35	164.84	96.81
129	Sal	Yes	117.18	125.47	105.52
136	Sal	Yes	179.28	146.84	116.63
142	Sal	Yes	177.35	138.75	102.90
143	Sal	Yes	117.26	155.14	109.44
130	TM/E	No	100.66	119.05	122.84
131	TM/E	No	138.41	179.32	122.50
134	TM/E	No	104.92	155.44	99.27
145	TM/E	No	124.40	206.05	96.99
147	TM/E	No	115.41	123.11	90.10
123	TM/E	Yes	123.93	163.33	238.75
124	TM/E	Yes	92.90	167.36	106.61
125	TM/E	Yes	193.32	174.24	110.34
133	TM/E	Yes	89.64	149.03	92.28
141	TM/E	Yes	119.65	182.82	106.71
146	TM/E	Yes	125.37	111.50	98.51

Table A-5. Raw data values for serum Cu (mg/L) concentrations in growing steers.

Id	Trt	Trt n	D 0	D 24	D 26	D 28	D 28.5	D 29	D 30	D 56
121	Sal	No	1.34	0.754	0.86	0.95	0.84	0.93	0.78	0.62
126	Sal	No	1.30	0.976	0.95	0.89	0.83	0.84	1.03	0.54
128	Sal	No	1.40	0.876	0.90	0.84	0.87	0.75	0.88	0.49
132	Sal	No	1.07	0.67	0.59	0.59	0.67	0.75	0.58	0.43
135	Sal	No	1.13	0.62	0.72	0.62	0.92	0.96	1.02	0.58
139	Sal	No	1.28	0.822	0.72	0.76	0.80	0.83	0.75	0.53
140	Sal	No	1.01	0.82	0.82	0.64	0.78	0.76	0.92	0.54
122	Sal	Yes	1.31	0.80	1.07	1.04	0.88	1.01	0.77	0.72
127	Sal	Yes	1.91	0.80	0.79	.	0.87	0.73	0.84	0.62
129	Sal	Yes	1.29	0.88	0.91	0.88	0.87	0.92	0.66	0.54
136	Sal	Yes	1.33	0.87	1.06	0.91	0.76	0.91	0.75	0.57
142	Sal	Yes	1.65	0.99	1.14	1.07	1.08	0.98	1.07	0.72
143	Sal	Yes	1.10	0.66	0.76	0.76	0.73	0.75	1.04	0.58
130	TM/E	No	1.16	0.93	0.96	1.23	0.74	0.89	0.65	0.58
131	TM/E	No	1.15	0.84	0.94	1.41	1.68	0.92	0.75	0.64
134	TM/E	No	1.19	0.94	1.03	0.83	0.86	1.33	0.72	0.57
145	TM/E	No	1.11	1.00	1.01	0.94	0.87	0.95	1.03	0.75
147	TM/E	No	1.16	0.95	1.01	0.95	0.93	1.24	1.30	0.60
123	TM/E	Yes	1.34	0.76	0.99	0.99	.	.	.	.
124	TM/E	Yes	1.32	1.02	1.30	1.12	1.16	1.40	1.68	0.73
125	TM/E	Yes	1.14	0.89	1.08	1.24	1.11	0.87	0.73	0.54
133	TM/E	Yes	1.10	0.82	1.08	0.93	1.02	0.90	1.20	0.67
141	TM/E	Yes	1.18	1.00	1.18	1.06	1.10	1.06	0.89	0.53
146	TM/E	Yes	1.35	0.98	1.23	1.12	1.16	1.27	0.95	0.94

Table A-6. Raw data values for serum Zn (mg/L) concentrations in growing steers.

Id	Trt	Tran	D 0	D 24	D 26	D 28	D 28.5	D 29	D 30	D 56
121	Sal	No	1.60	1.42	1.50	1.29	0.57	0.87	0.84	1.07
126	Sal	No	1.30	1.05	1.08	1.43	0.39	0.46	0.91	1.09
128	Sal	No	1.60	1.13	1.24	1.08	0.41	0.58	0.78	0.87
132	Sal	No	1.39	1.19	1.48	1.46	0.52	0.86	0.98	1.08
135	Sal	No	1.19	0.81	0.91	1.09	0.65	0.58	1.17	0.99
139	Sal	No	1.22	0.89	0.90	1.27	0.43	0.73	0.75	0.84
140	Sal	No	1.28	1.14	1.15	0.96	0.40	0.58	1.05	0.93
122	Sal	Yes	1.32	0.92	1.29	1.00	0.60	0.59	0.54	1.02
127	Sal	Yes	1.78	1.18	1.31	.	0.43	0.51	0.93	1.38
129	Sal	Yes	1.23	1.31	1.22	1.18	0.59	0.75	0.88	1.11
136	Sal	Yes	1.26	1.11	1.37	1.05	0.47	0.79	1.05	1.08
142	Sal	Yes	1.05	0.74	1.34	0.93	0.44	0.68	0.86	1.01
143	Sal	Yes	1.41	1.13	1.50	1.38	0.50	0.52	0.83	1.16
130	TM/E	No	1.32	1.02	1.41	1.48	0.55	0.84	0.96	1.12
131	TM/E	No	1.40	0.58	1.05	1.41	0.87	0.56	0.78	1.09
134	TM/E	No	1.38	0.68	1.20	1.36	0.53	0.78	0.92	1.06
145	TM/E	No	1.47	0.78	1.23	1.25	0.51	0.80	0.94	1.03
147	TM/E	No	1.19	0.85	1.07	1.23	0.57	1.01	0.99	0.96
123	TM/E	Yes	1.29	0.89	1.33	1.20	.	.	.	.
124	TM/E	Yes	1.33	0.67	1.22	1.15	0.75	0.47	0.60	0.93
125	TM/E	Yes	1.50	0.78	1.31	1.07	0.47	0.62	0.98	0.90
133	TM/E	Yes	1.24	0.76	1.04	1.09	0.51	0.50	0.73	1.00
141	TM/E	Yes	1.55	0.79	1.25	1.20	0.48	0.69	1.12	0.92
146	TM/E	Yes	1.48	0.92	1.25	1.20	0.81	1.33	0.92	1.16

Table A-7. Raw data values for whole blood selenium (ppb) concentrations in growing steers.

Id	Trt	Tran	D 0	D 24	D 26	D 28	D 28.5	D 29	D 30	D 56
121	Sal	No	101	330	247	307	252	135	286	185
126	Sal	No	106	248	188	91	250	225	225	229
128	Sal	No	103	225	282	121	73	228	292	224
132	Sal	No	73	243	265	210	246	271	105	210
135	Sal	No	74	117	131	245	225	315	278	254
139	Sal	No	116	99	200	234	120	213	212	219
140	Sal	No	135	103	332	279	204	200	192	127
122	Sal	Yes	124	288	81	255	.	66	261	223
127	Sal	Yes	71	229	239	61	225	250	276	293
129	Sal	Yes	77	287	253	308	.	187	206	207
136	Sal	Yes	141	98	270	216	226	211	228	223
142	Sal	Yes	161	221	334	139	110	59	102	325
143	Sal	Yes	136	226	171	221	250	65	172	235
130	TM/E	No	72	235	308	331	203	297	270	96
131	TM/E	No	86	255	273	377	142	161	223	113
134	TM/E	No	63	.	79	39	224	86	279	278
145	TM/E	No	95	61	116	90	256	89	210	140
147	TM/E	No	109	264	76	280	253	271	257	167
123	TM/E	Yes	129	.	.	191	.	.	.	.
124	TM/E	Yes	144	284	130	173	304	104	128	109
125	TM/E	Yes	115	281	81	111	281	188	304	194
133	TM/E	Yes	87	219	174	85	247	167	95	243
141	TM/E	Yes	96	191	352	296	287	47	79	284
146	TM/E	Yes	100	105	156	100	276	276	258	216

Table A-8. Raw data values for serum α-tocopherol (μg/mL) concentrations in growing steers.

Id	Trt	Tran	D 0	D 24	D 26	D 28	D 35	D 56
121	Sal	No	3.20	3.30	3.30	3.20	3.20	3.20
126	Sal	No	3.00	2.90	2.70	2.00	2.20	3.00
128	Sal	No	2.00	2.70	3.60	2.70	2.80	2.00
132	Sal	No	3.40	3.00	3.10	3.00	2.40	3.40
135	Sal	No	1.70	2.60	2.40	2.50	2.10	1.70
139	Sal	No	2.70	3.10	3.00	2.20	3.40	2.70
140	Sal	No	3.90	3.70	4.00	3.50	3.00	3.90
122	Sal	Yes	3.00	2.60	3.30	1.60	2.10	3.00
127	Sal	Yes	2.90	3.30	3.30	2.80	3.50	2.90
129	Sal	Yes	2.10	3.20	3.30	2.50	2.70	2.10
136	Sal	Yes	2.70	1.50	2.20	2.30	2.20	2.70
142	Sal	Yes	3.30	2.50	2.50	3.10	1.90	3.30
143	Sal	Yes	2.80	2.60	3.00	3.10	2.50	2.80
130	TM/E	No	3.30	9.30	6.80	4.40	4.00	3.30
131	TM/E	No	2.60	9.10	6.40	3.50	3.60	2.60
134	TM/E	No	2.20	7.90	3.90	3.70	3.90	2.20
145	TM/E	No	3.70	6.30	4.00	3.20	3.90	3.70
147	TM/E	No	3.80	8.60	4.90	3.20	3.00	3.80
123	TM/E	Yes	2.30	6.30	5.60	.	.	2.30
124	TM/E	Yes	2.50	7.80	6.50	2.80	2.30	2.50
125	TM/E	Yes	2.20	5.60	5.00	2.80	2.50	2.20
133	TM/E	Yes	3.70	10.20	6.20	2.90	4.10	3.70
141	TM/E	Yes	2.60	6.50	5.40	3.10	2.70	2.60
146	TM/E	Yes	3.20	6.60	4.50	3.20	3.00	3.20

Table A-9. Raw data values for serum γ-tocopherol (μg/mL) concentrations in growing steers.

Id	Trt	Tran	D 0	D 24	D 26	D 28	D 35	D 56
121	Sal	No	0.11	0.18	0.14	0.22	0.24	0.11
126	Sal	No	0.11	0.19	0.14	0.12	0.17	0.11
128	Sal	No	0.05	0.10	0.17	0.15	0.21	0.05
132	Sal	No	0.11	0.16	0.16	0.16	0.13	0.11
135	Sal	No	0.08	0.11	0.13	0.17	0.15	0.08
139	Sal	No	0.06	0.12	0.12	0.10	0.17	0.06
140	Sal	No	0.13	0.21	0.26	0.20	0.24	0.13
122	Sal	Yes	0.11	0.12	0.04	0.06	0.13	0.11
127	Sal	Yes	0.11	0.25	0.08	0.21	0.33	0.11
129	Sal	Yes	0.05	0.12	0.03	0.10	0.13	0.05
136	Sal	Yes	0.04	0.05	0.02	0.07	0.09	0.04
142	Sal	Yes	0.11	0.15	0.05	0.13	0.11	0.11
143	Sal	Yes	0.11	0.12	0.06	0.13	0.16	0.11
130	TM/E	No	0.11	0.05	0.08	0.11	0.15	0.11
131	TM/E	No	0.13	0.04	0.04	0.11	0.24	0.13
134	TM/E	No	0.07	0.05	0.05	0.13	0.14	0.07
145	TM/E	No	0.14	0.05	0.08	0.13	0.19	0.14
147	TM/E	No	0.12	0.05	0.06	0.13	0.11	0.12
123	TM/E	Yes	0.03	0.02	0.01	.	.	0.03
124	TM/E	Yes	0.13	0.05	0.02	0.05	0.13	0.13
125	TM/E	Yes	0.07	0.03	0.01	0.10	0.15	0.07
133	TM/E	Yes	0.15	0.10	0.02	0.07	0.24	0.15
141	TM/E	Yes	0.08	0.02	ND	0.13	0.14	0.08
146	TM/E	Yes	0.13	0.04	0.02	0.13	0.14	0.13

Table A-10. Raw data values for serum vitamin A (μg/mL) concentrations in growing steers.

Id	Trt	Tran	D 0	D 24	D 26	D 28	D 35	D 56
121	Sal	No	0.20	0.21	0.22	0.26	0.26	0.20
126	Sal	No	0.19	0.17	0.20	0.14	0.21	0.19
128	Sal	No	0.13	0.18	0.23	0.19	0.22	0.13
132	Sal	No	0.16	0.18	0.21	0.21	0.13	0.16
135	Sal	No	0.25	0.29	0.25	0.28	0.21	0.25
139	Sal	No	0.21	0.24	0.22	0.22	0.21	0.21
140	Sal	No	0.26	0.23	0.24	0.22	0.19	0.26
122	Sal	Yes	0.24	0.18	0.15	0.08	0.19	0.24
127	Sal	Yes	0.24	0.25	0.23	0.26	0.27	0.24
129	Sal	Yes	0.23	0.23	0.23	0.24	0.31	0.23
136	Sal	Yes	0.17	0.14	0.15	0.16	0.18	0.17
142	Sal	Yes	0.18	0.21	0.16	0.22	0.21	0.18
143	Sal	Yes	0.25	0.19	0.19	0.22	0.24	0.25
130	TM/E	No	0.26	0.69	0.17	0.22	0.22	0.26
131	TM/E	No	0.24	0.13	0.11	0.11	0.20	0.24
134	TM/E	No	0.26	0.10	0.15	0.22	0.21	0.26
145	TM/E	No	0.32	0.13	0.15	0.22	0.21	0.32
147	TM/E	No	0.22	0.10	0.13	0.23	0.19	0.22
123	TM/E	Yes	0.11	0.09	0.13	.	.	0.11
124	TM/E	Yes	0.32	0.09	0.11	0.10	0.21	0.32
125	TM/E	Yes	0.17	0.10	0.12	0.17	0.19	0.17
133	TM/E	Yes	0.24	0.09	0.10	0.09	0.21	0.24
141	TM/E	Yes	0.22	0.10	0.10	0.21	0.18	0.22
146	TM/E	Yes	0.25	0.10	0.11	0.23	0.18	0.25

Table A-11. Raw data values for lymphocyte proliferation to PHA (stimulation index.) in growing steers.

Id	Trt	Tran	D 0	D 7	D 28	D 35	D 56
121	Sal	No	47.12	89.50	10.50	0.65	13.75
126	Sal	No	0.06	57.57	0.08	2.16	7.55
128	Sal	No	1.97	12.25	99.58	1.23	0.73
132	Sal	No	0.33	0.98	0.73	1.51	2.36
135	Sal	No	2.85	66.98	0.29	1.29	4.00
139	Sal	No	0.76	1.09	0.73	1.82	0.64
140	Sal	No	11.66	2.26	4.43	3.09	2.97
122	Sal	Yes	1.99	0.64	1.00	0.70	2.18
127	Sal	Yes	0.94	2.56	167.93	0.95	1.54
129	Sal	Yes	0.80	1.05	2.76	5.98	1.86
136	Sal	Yes	0.04	78.71	2.35	1.47	2.23
142	Sal	Yes	1.69	1.67	1.39	1.23	0.82
143	Sal	Yes	0.86	7.92	2.25	1.56	3.36
130	TM/E	No	0.85	31.76	0.99	1.26	1.88
131	TM/E	No	2.39	1.41	1.35	0.81	3.73
134	TM/E	No	0.20	3.30	4.03	1.03	2.49
145	TM/E	No	2.43	17.70	1.03	0.93	0.83
147	TM/E	No	0.60	0.42	0.09	1.45	2.02
123	TM/E	Yes	1.79	9.36	3.11	.	.
124	TM/E	Yes	0.08	104.88	1.42	1.08	9.42
125	TM/E	Yes	0.40	28.45	0.68	3.11	2.08
133	TM/E	Yes	1.48	1.55	0.60	1.34	2.29
141	TM/E	Yes	1.76	14.51	0.13	1.05	1.63
146	TM/E	Yes	0.60	0.42	0.09	1.45	2.02

Table A-12. Raw data values for lymphocyte proliferation to OVA (stimulation index) in growing steers.

Id	Trt	Tran	D 0	D 7	D 28	D 35	D 56
121	Sal	No	1.05	1.08	2.07	0.84	1.90
126	Sal	No	0.04	1.20	0.12	1.29	0.97
128	Sal	No	0.34	1.26	0.88	2.60	0.83
132	Sal	No	0.42	0.38	0.72	1.00	2.10
135	Sal	No	0.60	2.51	0.11	0.83	1.09
139	Sal	No	0.31	0.41	0.52	1.07	0.87
140	Sal	No	1.31	0.68	4.06	0.99	1.12
122	Sal	Yes	1.21	0.34	1.16	0.73	0.69
127	Sal	Yes	0.51	0.88	5.34	0.81	2.56
129	Sal	Yes	0.60	0.44	1.63	5.62	0.95
136	Sal	Yes	0.08	1.92	1.27	1.39	0.88
142	Sal	Yes	0.80	1.26	0.25	1.04	0.77
143	Sal	Yes	0.46	4.35	0.87	1.26	1.81
130	TM/E	No	0.51	0.50	0.45	1.77	1.03
131	TM/E	No	0.68	0.83	0.20	0.95	1.33
134	TM/E	No	0.28	1.32	0.41	0.54	0.59
145	TM/E	No	0.36	1.01	0.53	0.41	0.83
147	TM/E	No	1.15	0.14	0.39	1.32	.
123	TM/E	Yes	1.29	2.90	1.69	.	.
124	TM/E	Yes	0.14	1.34	0.32	0.58	1.35
125	TM/E	Yes	0.69	1.44	0.86	1.14	0.97
133	TM/E	Yes	0.73	0.19	0.26	1.32	1.07
141	TM/E	Yes	0.53	0.72	0.07	0.95	0.94
146	TM/E	Yes	1.15	0.05	0.13	0.77	0.64

Table A-13. Raw data values for lymphocyte proliferation to KLH (stimulation index) in growing steers.

Id	Trt	Tran	D 0	D 7	D 28	D 35	D 56
121	Sal	No	2.05	0.63	1.73	0.39	1.57
126	Sal	No	0.08	1.69	0.12	2.02	0.95
128	Sal	No	0.62	0.86	3.03	0.91	1.02
132	Sal	No	0.19	1.08	1.16	1.47	0.60
135	Sal	No	1.11	2.08	0.15	1.09	5.28
139	Sal	No	0.75	1.91	0.42	1.15	0.75
140	Sal	No	1.71	1.06	2.18	1.24	1.15
122	Sal	Yes	1.09	0.71	0.81	1.06	0.82
127	Sal	Yes	0.74	1.03	2.80	0.69	0.93
129	Sal	Yes	1.14	0.48	2.54	5.97	1.20
136	Sal	Yes	0.03	1.85	2.64	1.18	1.08
142	Sal	Yes	0.64	0.95	0.48	0.78	0.69
143	Sal	Yes	1.37	3.21	0.89	1.13	1.68
130	TM/E	No	1.03	0.78	1.27	2.14	0.89
131	TM/E	No	0.98	0.63	0.83	1.00	1.36
134	TM/E	No	0.38	1.45	0.65	0.60	1.10
145	TM/E	No	0.72	1.44	0.70	0.50	0.82
147	TM/E	No	1.52	0.04	0.25	1.34	.
123	TM/E	Yes	0.96	3.84	1.64	.	.
124	TM/E	Yes	0.08	1.38	0.45	0.73	1.14
125	TM/E	Yes	1.69	0.79	0.80	0.70	1.29
133	TM/E	Yes	1.18	1.21	1.35	1.02	1.54
141	TM/E	Yes	1.23	0.46	0.25	0.95	0.68
146	TM/E	Yes	0.88	0.27	0.43	1.43	0.98

Table A-14. Raw data values of rectal temperature (ºC) following LPS inoculation in growing steers.

Id	Trt	Tran	H 0[†]	H 1	H 0.5	H 2	H 3	H 4
121	Sal	No	38.4	39.0	39.3	39.2	39.8	40.0
126	Sal	No	38.5	39.0	39.2	39.4	39.1	39.5
128	Sal	No	38.6	38.5	39.7	40.3	40.1	40.1
132	Sal	No	38.7	39.2	39.6	39.6	39.6	39.6
135	Sal	No	39.3	39.8	40.0	40.0	39.5	39.1
139	Sal	No	38.0	38.2	38.8	38.6	39.4	39.4
140	Sal	No	38.9	39.7	40.0	40.2	39.8	39.3
122	Sal	Yes	38.7	39.1	39.5	39.7	39.2	39.1
127	Sal	Yes	38.4	38.5	38.6	39.1	39.2	39.6
129	Sal	Yes	38.6	38.7	38.7	39.4	39.9	40.3
136	Sal	Yes	38.3	38.1	38.5	39.1	40.1	39.9
142	Sal	Yes	38.3	38.8	39.1	39.6	40.7	40.0
143	Sal	Yes	38.8	39.2	39.1	39.7	39.3	38.8
130	TM/E	No	39.3	39.7	40.1	40.2	40.7	40.3
131	TM/E	No	38.7	39.1	39.4	39.6	40.2	40.0
134	TM/E	No	38.8	39.0	39.7	39.8	40.4	40.3
145	TM/E	No	39.2	39.8	40.3	40.5	40.5	40.6
147	TM/E	No	40.1	40.3	40.3	40.6	41.1	41.0
123	TM/E	Yes	39.7	40.2	40.3	40.0	39.0	39.2
124	TM/E	Yes	39.0	39.3	39.4	39.8	39.6	39.3
125	TM/E	Yes	38.3	39.1	39.2	39.2	39.7	39.6
133	TM/E	Yes	38.7	39.0	38.7	39.9	39.6	39.7
141	TM/E	Yes	38.7	38.9	39.1	39.8	40.2	40.2
146	TM/E	Yes	38.9	39.2	39.7	39.8	40.4	40.0

[†]Hours post inoculation

Table A-14 (Cont.)

Id	Trt	Tran	H 6[†]	H 8	H 12	H 24	H 48
121	Sal	No	38.7	38.7	38.8	38.5	39.1
126	Sal	No	39.2	39.0	38.1	38.8	38.6
128	Sal	No	39.2	39.1	39.6	38.5	39.0
132	Sal	No	38.8	38.5	38.5	38.7	39.0
135	Sal	No	38.8	39.4	39.5	40.0	39.0
139	Sal	No	38.5	38.1	38.6	38.6	38.8
140	Sal	No	39.0	38.7	39.5	38.4	38.9
122	Sal	Yes	39.2	39.1	39.6	38.7	38.8
127	Sal	Yes	39.2	38.8	39.6	38.3	38.7
129	Sal	Yes	38.8	38.6	39.1	38.6	38.9
136	Sal	Yes	38.8	38.5	38.9	38.3	38.8
142	Sal	Yes	39.6	38.8	39.0	38.6	39.3
143	Sal	Yes	39.3	39.2	39.5	38.5	39.1
130	TM/E	No	38.9	38.6	39.1	39.3	39.3
131	TM/E	No	39.2	39.0	39.5	39.2	39.5
134	TM/E	No	39.0	39.0	39.0	38.8	39.4
145	TM/E	No	39.3	39.5	39.5	39.2	39.8
147	TM/E	No	39.5	39.5	39.5	39.5	39.7
123	TM/E	Yes	38.6	.	.	.	.
124	TM/E	Yes	39.7	39.2	41.1	38.8	38.7
125	TM/E	Yes	S	38.7	39.3	39.2	38.8
133	TM/E	Yes	38.5	38.8	39.3	38.4	39.2
141	TM/E	Yes	39.0	38.5	38.9	39.1	39.0
146	TM/E	Yes	39.0	38.8	39.1	39.4	39.3

[†]Hours post inoculation

Table A-15. Raw data values for humoral antibody titer to KLH (% positivity) concentrations in growing steers.

Id	Trt	Tran	D 0	D 14	D 21	D 35	D 42	D 49	D 56	D 63
121	Sal	No	50.26	40.92	33.26	42.13	57.03	47.21	50.52	45.76
126	Sal	No	45.83	44.55	41.05	45.28	55.47	47.54	59.64	43.16
128	Sal	No	40.63	46.73	47.05	41.65	38.02	47.86	54.95	47.70
132	Sal	No	65.10	56.42	64.74	51.82	66.93	72.85	73.96	67.50
135	Sal	No	50.52	63.92	43.97	39.47	64.58	61.17	68.75	62.14
139	Sal	No	51.56	49.39	44.13	48.67	60.94	63.76	57.29	34.23
140	Sal	No	50.52	62.23	41.37	73.85	68.75	63.60	76.30	44.13
122	Sal	Yes	66.41	55.45	67.17	53.51	58.07	76.74	70.31	72.15
127	Sal	Yes	52.60	48.43	44.13	48.67	60.68	59.22	33.90	55.00
129	Sal	Yes	56.77	47.46	40.24	52.78	52.08	70.09	55.99	54.00
136	Sal	Yes	42.45	36.56	45.59	65.38	60.68	55.81	53.39	48.67
142	Sal	Yes	44.79	40.68	38.62	36.56	50.52	49.32	50.00	58.60
143	Sal	Yes	66.15	49.64	50.95	44.55	53.13	74.96	108.1	59.08
130	TM/E	No	51.04	50.61	43.81	48.91	56.51	61.82	58.33	74.82
131	TM/E	No	33.59	52.06	38.94	46.97	59.64	67.82	61.20	45.59
134	TM/E	No	46.61	55.45	40.89	55.21	51.56	67.33	55.73	55.98
145	TM/E	No	50.00	53.27	44.94	50.61	52.86	57.27	53.65	47.22
147	TM/E	No	57.81	57.14	37.64	46.73	62.50	56.79	55.21	38.94
123	TM/E	Yes	38.02	53.51	42.02	.	.	.	.	.
124	TM/E	Yes	59.90	55.21	40.89	55.69	69.79	61.17	56.51	55.69
125	TM/E	Yes	44.27	54.72	43.00	46.00	72.66	59.38	52.60	38.29
133	TM/E	Yes	48.70	66.34	42.18	57.14	63.54	85.34	72.14	56.95
141	TM/E	Yes	47.92	44.79	36.67	41.40	54.69	71.71	58.59	46.97
146	TM/E	Yes	40.10	44.55	56.30	56.66	69.79	76.74	60.16	74.82

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